Publication Number 366

AMERICAN LECTURE SERIES®

A Monograph in

The BANNERSTONE DIVISION *of*
AMERICAN LECTURES IN PHYSIOLOGY

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The Physiological Basis Of Diuretic Therapy

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CHARLES C THOMAS • PUBLISHER

Springfield • Illinois • U.S.A.

CHARLES C THOMAS • PUBLISHER
BANNERSTONE HOUSE
301-327 East Lawrence Avenue, Springfield, Illinois, U.S.A.

Published simultaneously in the British Commonwealth of Nations by
BLACKWELL SCIENTIFIC PUBLICATIONS, LTD., OXFORD, ENGLAND

Published simultaneously in Canada by
THE RYERSON PRESS, TORONTO

Library of Congress Catalog Card Number 59-11906

Printed in the United States of America

DEDICATION

To Homer W. Smith, who as teacher and critic guided my early investigative efforts, and whose methods and concepts have formed the foundation of my subsequent work.

INTRODUCTION

At the beginning of the present century the practice of internal medicine was largely an empiric art. Few specific drugs were available; therapy was largely supportive and symptomatic. Digitalis or squill, xanthines or potassium chloride, and fluid restriction coupled with purgation by calomel constituted the elements of the treatment of dropsy. Today the physician has at his disposal far more effective methods for managing the edematous patient than he had half a century ago. He has purified digitalis glycosides for the treatment of myocardial decompensation; he has salt free prepared foods and cation exchange resins as means of limiting sodium intake, and he has a variety of potent diuretic drugs to facilitate the elimination of edema. However, of even more significance, he has or should have at his command a fuller understanding of renal physiology, a more complete knowledge of the patho-physiology of edema, and a better appreciation of the possible mechanisms of diuretic action than had his counterpart of 50 years ago.

The average practitioner, even the undergraduate medical student, lacks the time to read the voluminous literature on renal physiology, on fluid and electrolyte metabolism, and on the mechanisms of diuretic drug action, much less to digest and assimilate it. As a consequence, he tends to practice somewhat empirically. No matter how good the therapeutic result may be, he misses the excitement and intellectual satisfaction which comes from rational therapy, rationally applied.

Part I of this monograph has been written to provide a background of basic information concerning the distribution of water and electrolytes within the body, the mechanisms of regulation of volume and composition of the body fluids, and the abnormalities of function which result in the formation of edema. Because a number of excellent books on volume and composition of the body fluids have appeared recently, these subjects are treated briefly and only to the extent necessary to provide a basis for discussion

of regulatory mechanisms, their derangements in edema, and the modes of action of diuretics. In contrast, renal mechanisms of regulation of the salt and water content of the body are much more intensively treated, for a number of revolutionary new concepts have been developed in recent years which are as yet unfamiliar to the majority of students and practitioners of medicine. The author has attempted to integrate these views and to develop a logical and understandable description both of normal function and of the abnormalities of function which result in the formation of edema.

Part II of this monograph begins with a functional classification of diuretics and continues with a discussion of the mechanism of action of each in sequence. While greatest emphasis is accorded those agents which are at present clinically most useful, some attention is directed to those which may prove to be harbingers of the diuretics of the future as well as to those which have contributed uniquely to an understanding of function in the past. Part II concludes with a brief discussion of certain general complications of diuretic therapy.

This monograph is neither an exhaustive review of the literature nor an empiric guide to therapy. It tells a story in the somewhat one sided way the author sees it; it may therefore irritate the expert. In spots it is difficult reading, for as knowledge expands, concepts become more involved; it will appeal to some as overly academic. It is hoped that it will help the student, whether practitioner or undergradute, to understand the problem of and to gain a working knowledge of the physiologic basis of diuretic therapy. It will entirely satisfy no one, least of all the author, for the story is incomplete.

Because this monograph has been written for the student rather than for the investigator, citations have been restricted to a selected few articles, frequently reviews. From these sources, a more extensive bibliography may be developed by those who wish to delve more deeply into any aspect of the subject. To assist the more casual reader, a brief summary has been provided at the end of each chapter.

ACKNOWLEDGMENT

I am indebted to my associates of the past several years for much of the substance of this monograph and for many of the concepts developed in its pages. I am more immediately indebted to Drs. Richard H. Kessler, Gerhard Giebisch, and Roy C. Swan who have read and criticized portions or all of the manuscript. I am likewise indebted to the National Heart Institute of the National Institutes of Health and to the Life Insurance Medical Research Fund for long term and generous support of my investigations. Finally, I am indebted to the authors and journals noted in the figure legends for permission to reproduce certain illustrative matter.
permission to reproduce certain illustrative matter,
and to Miss Priscilla Dumschott for cheerful assistance in the preparation of the manuscript.

R.F.P.

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THE PHYSIOLOGICAL BASIS OF DIURETIC THERAPY

Part 1

Volume, Composition and Mechanisms of Homeostasis of Body Fluids; Abnormalities in Edema

Chapter I

BODY WATER COMPARTMENTS

Total Body Water. The water content of the normal human body varies between 45 and 70 per cent of body weight. The larger figure applies to the infant, the smaller to the obese adult. A range of 50 to 60 per cent would include most normal adults. Since adipose tissue and bone contain relatively little water while muscle contains over 70 per cent, it is obvious that leanness and hyposthenic habitus favor high, and obesity and sthenic habitus favor low total body water content. Body water can be considered as being distributed among three compartments: an *extracellular*, an *intracellular* and a *transcellular compartment* (see Fig. 1).

VOLUME OF COMPARTMENTS

The Extracellular Compartment may be subdivided into a vascular or plasma compartment of ± 4 per cent and an interstitial compartment of ± 16 per cent of body weight.[1] Lymph is generally included in interstitial fluid, there being no means of distinguishing the two moieties, and probably represents 2 to 3 per cent of the body weight. Fluid in the plasma compartment circulates rapidly through vascular channels, while that in the interstitial compartment diffuses more slowly through tissue interstices Claude Bernard more than a century ago pointed out that interstitial fluid constitutes the true environment of the body, in that it bathes all tissue cells, supplies their nutriments, and removes their wastes. Exchanges between plasma and interstitial fluid occur with great rapidity across the single layered endothelium of the capillary wall. Because the volume of interstitial fluid is normally small in com-

[1]Some maintain that connective tissue constitutes a third subdivision of the extracellular compartment one with which chloride ions freely exchange, but one from which certain other solutes are excluded. For the purpose of our discussion, the simpler definition of the extracellular compartment, given above, is adequate.

of body weight in the fasting state; according to Edelman, the lower value is more nearly correct in man.

METHODS EMPLOYED IN ESTIMATING VOLUMES OF BODY FLUID COMPARTMENTS

Total body water, plasma volume, and extracellular fluid volume in man are measured directly, although with variable precision, by dilution techniques. Interstitial fluid volume may be calculated as the difference between total extracellular volume and plasma volume. Intracellular fluid volume may be similarly calculated as the difference between total body water and the sum of transcellular and extracellular volumes. The two calculated volumes, namely interstitial and intracellular, are subject to the sum of the errors and uncertainties of the component measurements. Transcellular fluid volume is subject only to an educated guess.

Total Body Water. Of the several body fluid compartments of man, total body water can be measured with greatest precision. Two isotopes of water, D_2O (deuterium oxide) and T_2O (tritium oxide), when administered parenterally or per os, distribute uniformly throughout all fluid compartments of the body according to their water content. When given intravenously, these isotopes diffuse rapidly across capillary walls, cell membranes and all epithelial barriers to reach 90 per cent of equilibrium with body water within 30 minutes and virtually complete equilibrium within 2 to 3 hours. Even in edematous patients, distribution is relatively rapid.

One can calculate the volume of distribution of D_2O or T_2O, i.e., the total volume of body water, according to the following equation:

$$\text{Volume of Distribution} = \frac{(\text{Quantity Administered} - \text{Quantity Excreted}) \times 100}{\text{Concentration in Plasma Water (per cent)}}$$

This equation is a general one, and depending on the properties of the substance introduced into the body, can be used to measure total body water, plasma volume, or extracellular fluid volume. As an example of its use in measuring total body water, let us inject 100 ml. of D_2O as isotonic saline intravenously into a normal man

parison with that of cells, it introduces little delay in the diffusion of materials between plasma and cells.

The Intracellular Compartment is neither a single nor a homogenous compartment, but represents, in the aggregate, the sum of the fluid contents of all the cells of the body. Since liver cells, fat cells, and muscle cells vary greatly in water content and in chemical composition, one must look somewhat askance at a simplified representation of this compartment such as that shown in Figure 1. Intracellular water amounts on an average to 30 to 35 per cent of body weight.

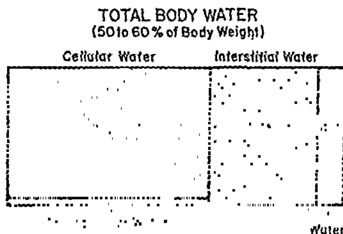


Fig 1

The Transcellular Compartment includes several discontinuous moieties of differing compositions, including the digestive secretions, the cerebrospinal and intraoccular fluids, and synovial, pleural and peritoneal fluids. The common factor which justifies the designation of a transcellular compartment is the fact that each portion of fluid, although extracellular, is separated from the blood plasma not only by the endothelium of the capillary wall but also by a continuous layer of epithelial cells. In varying degree this cell layer modifies the composition of transcellular fluid with respect to that of extracellular fluid. Normally the volume of the transcellular compartment is relatively small, the only subdivision having quantitative significance being that of the digestive secretions. These latter have been variously estimated as 1 to 6 per cent

various organs. Radioiodinated plasma albumin injected intravenously distributes exactly as does Evans blue, for both are dependant on the volume of distribution of the plasma proteins. A choice between the two methods depends solely on technical preference.

Plasma volume may also be estimated from measurements of whole blood volume and hematocrit. Whole blood volume can be measured as the volume of distribution of red cells labelled with radioactive chromium-51. Red cells certainly are better retained within the vascular tree than are plasma proteins, yet their sequestration within the spleen is a possible source of error. Another and more significant error inherent in the red cell distribution method derives from the fact that the hematocrit of blood in the terminal vascular bed of most organs is distinctly less than that in the blood contained in arteries and veins supplying them. Hence hematocrits and volumes of red cell distribution based on venous or arterial blood samples underestimate plasma volume. While the two methods do not agree precisely, either the volume of distribution of labelled protein or of labelled red cells provides useful information concerning plasma volume.

Extracellular Fluid Volume. For studies on edema and the action of diuretics a precise measure of extracellular fluid volume is most to be desired. Unfortunately, the various methods currently in use give widely divergent values, ranging from 16 to 30 per cent of body weight in normal man. The ideal substance to measure extracellular fluid volume should have the following properties:

(1) It should diffuse rapidly and readily from the vascular bed throughout the interstitial fluid spaces of all tissues and reach equilibrium concentrations in all moieties of extracellular water within a reasonable period of time.

(2) It should be absolutely and completely excluded from cells.

(3) It should not bind to plasma proteins or be adsorbed on collagen fibers or on bone crystal surfaces.

(4) Its rate of excretion should be low in comparison with the rate at which it distributes in the extracellular phase.

(5) It should be completely inert in the body; i.e., not metabolized and non-toxic.

weighing 90 Kg. After an equilibration period of two hours, a blood sample is drawn and the plasma separated and analyzed. The D_2O[2] concentration is found to be 0.200 volumes per cent of the plasma water (0.2 ml. of D_2O per 100 ml. of plasma water). During a two hour equilibration period, urinary, respiratory, and cutaneous losses have been found to average 0.4 per cent of the administered dose. Substituting in the equation given above:

$$\text{Volume of Distribution} = \frac{(100 - 0.4) \times 100}{0.200} = 49{,}800 \text{ ml. or } 49.8 \text{ liters}$$

Since our subject weighed 90 Kg., body water constitutes $\frac{49.8}{90} \times 100 = 55.3$ per cent of body weight. Although the techniques for quantifying D_2O and T_2O are remarkably precise, with errors of $\pm$ 1.0 per cent, the overall accuracy of the measurement of total body water is appreciably less. Thus deuterium exchanges with certain labile hydrogen atoms of proteins and carbohydrates, the net effect of which is to increase the apparent volume of distribution of D_2O by one to three per cent of body weight above its true value. Although the measurement of total body water by the isotope dilution method is relatively precise, the possible error is a liter or more. Accordingly, changes in body water over short periods of time can be estimated more precisely from changes in body weight than from changes in volume of D_2O distribution.

Plasma Volume may be estimated by measuring the volume of distribution of materials which are largely retained within the vascular compartment when they are injected intravenously. The dye Evans blue binds firmly to the plasma proteins, hence is retained within the vascular system to whatever extent the proteins are retained. Lymph from muscle contains a small amount of protein, whereas that from the liver is rich in protein. Accordingly, Evans blue is found in low concentration in muscle lymph and in much higher concentration in liver lymph. The volume of distribution of the dye, calculated by means of the equation given for total body water, somewhat overestimates plasma volume, for it is impossible to measure the amount of dye present in the lymph of

[2]D_2O when mixed with body water becomes largely DHO. It simplifies the calculation of volume of distribution and introduces no error to consider it as D_2O

advanced disease, composition as well as volume may be altered. *Early or in mild disease, edema collects in dependent parts of the* body. Later it becomes generalized. Two kilograms, or about 5 lb. of excess interstitial fluid is the least that can be recognized with certainty by the "pitting" which occurs on pressure over the tibia. In its most severe form represented by anasarca and/or massive ascites, edema fluid may collect to the extent of 50 lb. or more. Strictly speaking, ascites represents an expansion of the peritoneal moiety of transcellular fluid. The composition of ascitic fluid is not essentially different from that of edema fluid except for its high protein content. It is no doubt a transudate of intrahepatic and portal capillaries, modified to some extent by the peritoneal epithelium. Relative isolation from lymphatic and vascular channels makes its mobilization more difficult. However, for the purposes of our discussion, ascitic fluid and edema fluid may be considered together as biochemical and functional equivalents.

Disturbances of Function in Edema. Tissue functions are disturbed by edema in no less than two ways. First, an excess of interstitial fluid, rendering tissues boggy and turgid, interferes mechanically with their functions. For example, digestive disturbances, which are commonly associated with edema, are presumably due in part to altered gut motility. The most common complaints of edematous patients are related to swelling of the ankles, protuberance of the abdomen, and gain in weight, all of which have their adverse mechanical as well as cosmetic implications. Second, an excess of interstitial fluid constitutes a stagnant pool interposed between circulating blood plasma and tissue cells. Diffusion distances are increased and, as a consequence, the concentrations of nutriments are lower and of wastes higher within and immediately surrounding cells. Because the environment is less favorable, cell function may be disturbed. Pulmonary edema constitutes a special threat in that it reduces lung compliance, interferes with ventilation of the alveoli, and serves as a barrier to exchange of gases between alveolar spaces and blood.

(6) It should be readily determinable in low concentration in plasma with a high degree of precision. Needless to say, no such substance is known at the present time. However, were one to exist, its volume of distribution, calculated from the general equation given for total body water, would give a precise measure of extracellular volume.

It is the author's opinion that radiosulfur-35 as sulfate ion exhibits more of the ideal characteristics outlined above than any other of the several substances proposed. In nephrectomized dogs where one can eliminate the disturbing element of renal excretion, Swan *et al.* have shown that radiosulfate, mannitol, and thiosulfate are distributed in equal volumes, averaging about 22 per cent of body weight. In the presence of normal renal function, rapid excretion of both mannitol and thiosulfate render measurements of extracellular volume by direct dilution methods suspect. Radiosulfate is much more slowly excreted and less rapidly metabolized than thiosulfate and mannitol, hence preferable for measurement of extracellular volume.

It is possible to avoid the complication of high rate of excretion of mannitol, thiosulfate, and other substances whose volumes of distribution have been proposed as measures of extracellular fluid in the following way. The substance is infused intravenously at a constant rate until contancy of plasma concentration is achieved and until diffusion equilibrium between plasma and interstitial fluid is attained. Two to six hours or more may be required. At this time a blood sample is drawn and the concentration of the substance in plasma water is determined. The bladder is emptied and the infusion stopped. All urine is collected until the excretion of the substance in question is complete. The total quantity excreted, divided by the concentration in plasma water at the time the infusion was stopped, gives the apparent volume of distribution. The method is laborious, time consuming and subject to errors which render it no more accurate than the simpler radiosulfate method.

Changes in Interstitial Volume in Edema. Edema represents an abnormal accumulation of interstitial fluid. In its more common form, the ionic composition and osmotic pressure of this fluid are essentially normal, only volume is increased. However, in more

Chapter II

IONIC COMPOSITION OF BODY FLUIDS

IN discussing the composition of the body fluids, it is necessary to utilize the terminology of chemical equivalents,[*] familiar enough to recent graduates of medicine, but perhaps sufficiently unfamiliar to the more mature to justify brief explanation. When the concentration of each ionic constituent of a complex solution like interstitial fluid is expressed in milliequivalents (mEq.) per liter, the sum of the concentrations of all the positive ions (cations) such as sodium, potassium, calcium, and magnesium exactly equals the sum of the concentrations of all the negative ions (anions) such as chloride, bicarbonate, sulfate, phosphate and protein. Balance in terms of equivalents is obligatory, for solutions must be electricallly neutral; each positive ionic charge must be balanced by a negative ionic charge. Balance in terms of weights per unit volume (milligram per cent) is impossible to achieve.

Definition of Chemical Equivalents. A milliequivalent (mEq.) is 1/1000th of an equivalent. Ion concentrations in body fluids, when expressed in mEq., are whole numbers, hence are more convenient to handle than if expressed in equivalents. One milligram

[*]On a trip to Scotland some years ago, my wife and I stopped at a small hotel in the highlands. Leaving early, I anticipated the usual delay in toting up the bill for bar, dinner, bed and breakfast. Instead, the desk clerk, after a few preliminary scratches, promptly quoted to the exact penny the figure I had laborously worked out earlier that morning On inquiry as to the method employed, he stated that it was really quite simple "I convert pounds and shillings to dollars, add up the bill in dollars and reconvert to pounds and shillings." Treating chemical composition in any terms other than that of equivalents involves a system of conversions, calculations, and reconversions considerably more formidable than those required by the Scottish currency

SUMMARY

Water constitutes some 50 to 60 per cent of the weight of the normal human body. This water is distributed among a plasma compartment (4 per cent), an interstitial compartment (16 per cent), a transcellular compartment (1 to 6 per cent), and a cellular compartment (30 to 35 per cent) of body weight. Edema represents primarily an abnormal expansion of volume of interstitial fluid; composition may be entirely normal. Edema interferes mechanically with tissue functions and hinders exchange of nutriments and wastes between blood and tissue cells. In the lung, edema reduces pulmonary compliance, interferes with alveolar ventilation and restricts diffusion of gases between blood and alveolar spaces.

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stitial fluid and plasma are real, it is apparent from Table I that they are not large. As a first approximation, they can be neglected and interstitial fluid can be assumed to have the ionic structure of plasma.

IONIC CONSTITUTION OF INTRACELLULAR FLUID

Owing to several difficulties, knowledge of the ionic composition of intracellular fluid is far less exact than is that of extracellular fluid. It is impossible to analyze cell contents directly. Tissues must be analyzed and cell composition calculated from total minus extracellular ionic concentrations. Since the volume of the extracellular phase of tissues cannot be determined with any degree of certainty, both intracellular volume and composition can only be approximated. Furthermore, the various tissues of the body differ in their protein, lipid and water contents and no doubt in their ionic constitutions as well. One cannot, therefore, describe a general chemical structure of intracellular fluid applicable to all cells. Finally, one cannot determine the physical state within the living cell of some of the electrolytes, whether bound or free, whether ionized or un-ionized.

Certain features which are characteristic of intracellular fluids and which serve *to distinguish them from extracellular fluids* are evident in the observations on skeletal muscle summarized in Table II. The major intracellular cations are potassium and magnesium. Relatively little sodium, the major extracellular cation, exists in

TABLE II

Ionic Structure of Muscle

	mEq/L. H_2O
Na	10
K	160
Mg	35
Cl	2
Protein	55
PO_4 (organic)	140

TABLE I

Ionic Structure of Plasma and Interstitial Fluid

	Plasma	Plasma Water	Interstitial Fluid
	(mEq./L.*)	(mEq /L.*)	(mEq /L water†)
Na	142	151	144
K	4	4.3	4 0
Ca	5	5 4	2.5
Mg	3	3.2	1.5
ΣCation	154	163 9	152 0
Cl	103	109 7	114
HCO_3	27	28.7	30
PO_4	2	2.1	2.0
SO_4	1	1.1	1 0
Organic Acid	5	5.3	5.0
Protein	16	17	0 0
ΣAnions	154	163.9	152.0

*Average values

†Rough approximations, calculated

present in somewhat greater concentrations to take their place. (b) The sum of all cations must equal the sum of all anions both in interstitial fluid and in plasma. (c) The osmotic pressure (dependent on numbers of solute particles) of interstitial fluid must equal that of plasma, except for the slight colloid osmotic (oncotic) force due to the presence of proteins in plasma. This oncotic force is balanced by a hydrostatic force, the capillary blood pressure. As is evident from Table I, the diffusible (non-protein) anions of interstitial fluid are slightly more concentrated than are those of plasma water. In contrast the diffusible cations are slightly less concentrated than are those of plasma water. Calcium and magnesium are considerably lower in concentration in interstitial fluid because *they are in part bound to proteins of the plasma,* hence are non diffusible. Although the differences in ionic composition of inter-

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*Average values

†Rough approximations, calculated

present in somewhat greater concentrations to take their place. (b) The sum of all cations must equal the sum of all anions both in interstitial fluid and in plasma. (c) The osmotic pressure (dependent on numbers of solute particles) of interstitial fluid must equal that of plasma, except for the slight colloid osmotic (oncotic) force due to the presence of proteins in plasma. This oncotic force is balanced by a hydrostatic force, the capillary blood pressure. As is evident from Table I, the diffusible (non-protein) anions of interstitial fluid are slightly more concentrated than are those of plasma water. In contrast the diffusible cations are slightly less concentrated than are those of plasma water. Calcium and magnesium are considerably lower in concentration in interstitial fluid because they are in part bound to proteins of the plasma, hence are non diffusible. Although the differences in ionic composition of inter-

reactivate the cell pumps and restore normal cellular and plasma concentrations.

Figure 3 A and B illustrate the general concepts of Glynn, Shaw, Hodgkin, Keynes and others of the ion pump of erythrocytes, muscle cells and nerve cells. The passive movements of sodium and potassium into and out of cells are shown by the straight arrows to either side of Figure 3A. The passive diffusion of sodium into the cell (sodium influx, heavy arrow) exceeds the passive diffusion out of cell (sodium efflux) for it occurs downhill along a large concentration gradient. In contrast, passive efflux of potassium exceeds passive influx for the concentration gradient is the reverse of that for sodium. Were passive influx of sodium and passive efflux of potassium to continue unopposed, concentrations of the two ion species inside and outside the cell would equalize. The curved arrows connected by a circle represent the active fluxes which oppose the attainment of diffusion equilibrium. Sodium is pumped out of the cell, potassium is pumped into the cell, and the two processes seem to be linked. But little is known of the energetics of this coupled transport system, other than the fact that it utilizes phosphate bond energy.

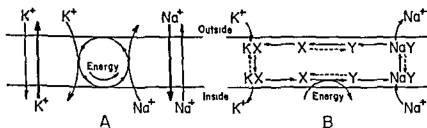


Fig 3. Mechanisms of transport of sodium and potassium across cell membranes. A. Straight arrows, passive diffusion of ions. Curved arrows, active transport of ions. B. Hypothetical coupled carrier mechanism which might actively transport potassium into cells and eject sodium from cells (From I. M Glynn. *J Physiol.* *134*:278, 1956.)

Cell membranes in general are polarized. negative inside, positive outside, and this polarization seems in many instances to be related to the difference in potassium concentration on the two sides of the membrane. The potential difference (P.D.) across many mam-

muscle cells. The major intracellular anions are organic phosphates (adenosine mono-, di-, and triphosphates, glycerophosphate, creatine phosphate) and proteins. Relatively little chloride, the major extracellular anion, exisits in muscle cells.[7] Although muscle has been used to illustrate the marked differences in composition which distinguish intracellular from extracellular fluids, other cell types exhibit differences which are qualitatively similar.

The total osmolal concentration of intracellular fluid is thought to be essentially the same as that of extracellular fluid.[8] However, this fact cannot be derived from the data given in Tables I and II, for the osmotic activity of the polyvalent ions, magnesium, organic phosphates and protein, is unknown. It is even impossible to balance total cations against total anions for the concentrations of a number of components are uncertain. However, exact ionic balance must exist.

Maintenance of Differences in Ionic Composition. The marked differences in the concentrations of sodium, potassium, and chloride between cell contents and their surrounding fluid environment do not derive from any absolute ionic impermeability of cell membranes. The radioisotopes of these ions, introduced into extracellular fluid, exchange more or less rapidly with their non-radioactive counterparts within cells. One is forced to conclude that the tendency of sodium ions to diffuse into cells and of potassium ions to diffuse out along their concentration gradients must be counteracted by the active outward pumping of sodium ions with or without the active inward pumping of potassium ions. It is probable that transport of both ions is active.

These ion pumps are metabolically activated. Cold and certain metabolic inhibitors inactivate the pumps. The most familiar example of the changes in cell composition which occur when the ion pumps fail is the loss of red blood cell potassium to plasma and the entry of plasma sodium into red blood cells when bank blood is stored in the cold. Rewarming the blood and adding glucose

[7]Certain cells contain more chloride than muscle, e.g., gastric mucosa, kidney, skin, testis, ovary and red blood cells.

[8]The view of Robinson, Opie and others that cells are hypertonic to their surroundings has not been generally accepted.

limiting value, the system will operate in counter clockwise direction, pumping sodium out of and potassium into the cell.

The distribution of chloride (low inside the muscle cell, high outside) is that predicted by the Gibbs-Donnan rule and is analogous to the distribution already described between plasma and interstitial fluid. The difference in chloride concentration across the cell membrane is, however, far greater than that across the capillary membrane, for the anions of the intracellular fluid are largely non-diffusible, whereas those of plasma, except for protein, are largely diffusible. The concentration of chloride within the cell must be low to satisfy the requirements that total anions must equal total cations and that total osmolal concentration of intracellular fluid must equal that of interstitial fluid. If the potassium content of cells is increased acutely as it is by the infusion of potassium salts, their chloride content increases. Conversely those cells in which the chloride concentration is normally high (gastric mucosa, gonad, etc.) must exhibit lower intracellular concentrations of non-diffusible anions.

Little is known concerning the bicarbonate ion content of muscle and most tissue cells, largely because of uncertainty in partitioning the carbon dioxide which can be extracted from them among the several forms in which it is known to exist: dissolved carbon dioxide and carbonic acid, carbamino bound carbon dioxide, and bicarbonate ion. Nearly all would accept the thesis that cells are freely permeable to dissolved carbon dioxide. If true, the concentration of dissolved carbon dioxide and of carbonic acid will be essentially the same in cell water and in the water of blood plasma and interstitial fluid, namely 1.2 to 1.4 mEq. per liter. Opinion varies as to the relative proportion of the remainder of the carbon dioxide (6 to 10 mEq. per liter) present as bicarbonate ion and in carbamino combination. If present largely in carbamino combination, bicarbonate ion concentration and cell pH will be low. If present largely in the form of bicarbonate, cell pH will approach neutrality. If cell bicarbonate concentration and pH are low (~pH 6), it is possible that the cell membrane is relatively permeable to bicarbonate ion and that low internal bicarbonate concentration is an expression of a Donnan ion distribution similar to

malian cell membranes can be shown to vary with the concentration of potassium in the interstitial fluid in the following manner:

$$\text{P.D (millivolts)} = 61 \times \log \frac{[^{K}\text{cellular}]}{[^{K}\text{interstitial}]}$$

The P.D. can be considered as a potassium diffusion potential, the origin of which is explained in the following manner. The cell membrane is absolutely impermeable to the large polyvalent protein and organic phosphate anions of the cell contents. The cell is effectively, although not actually impermeable to sodium by virtue of the operation of membrane pumps which continuously eject the sodium which diffuses in. In essence the cell membrane can be considered to be relatively permeable only to potassium ions and perhaps to chloride ions. Positively charged potassium ions tend to diffuse out of the cell, downhill along their concentration gradient. They are restrained by the increasing negative charge left within the cell. A state is reached at which the outward diffusion of potassium, driven by concentration difference, is just balanced by increasing cellular negativity, restraining further diffusion. This state is described by the equation given above. If cellular concentration of potassium were ten times interstitial concentration, the P.D. would be 61 mv. (log 10 = 1). If cellular and interstitial concentrations were equal, the P.D. would be 0.0 mv. (log 1 = 0). Values of P.D. for most mammalian cells vary from 60 to 90 mv., corresponding to a ratio of cellular/interstitial potassium concentration of 10/1 to 30/1.

Figure 3B illustrates a hypothetical cyclical carrier system advanced to explain the active coupled ion fluxes in red cells, muscle and nerve. It is postulated that K^+ and Na^+ cross the cell membrane in combination with the carriers X and Y; X is K^+ specific; Y is Na^+ specific. The complexes KX and NaY are presumed to be freely diffusible within the substance of the membrane and in equilibruim with K^+ and X and with Na^+ and Y, respectively, at the inner and outer surfaces of the membrane. At the inner surface of the membrane, X is converted to Y by the expenditure of phosphate bond energy. So long as energy is supplied, and so long as the concentrations of Na^+ inside and of K^+ outside are above some

limiting value, the system will operate in counter clockwise direction, pumping sodium out of and potassium into the cell.

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that for chloride. If cell bicarbonate concentration and pH are relatively high (~pH 7), it is possible that the cell membrane is considerably less permeable to bicarbonate than to chloride ion or that there is some active mechanism which regulates cell pH.

OSMOTIC PRESSURE AND DISTRIBUTION OF WATER

Osmotic forces are of such importance in determining the distribution of water among the several fluid compartments of the body that it is important to have a clear appreciation of their mode of operation.

When a solution and pure solvent are separated by a semipermeable membrane, the solvent passes into the solution by a process known as osmosis. The osmotic pressure is that hydrostatic pressure which must be applied to the solution to prevent this inward migration of solvent (see Fig. 4). Cell membranes in general are more or less permeable to water but effectively impermeable to many crystalloidal solutes such as sodium, chloride and bicarbonate, hence may be classified as basically semipermeable. By effective impermeability is meant extrusion of a substance from a cell as rapidly as it enters under electrical and chemical forces.

When red blood cells are placed in distilled water, water enters by osmosis and the cells swell and hemolyze. If the cells are placed in 0.9 per cent saline, they undergo no change in volume. The osmolal concentration of the saline exactly equals that of the cell contents, i.e., the two are isosmotic. Since no osmotic swelling or shrinkage occurs, the saline is said to be isotonic.

A 1.8 per cent solution of urea has the same osmolal concentration as a 0.9 per cent solution of sodium chloride, i.e., the solutions are isosmotic. However, when red cells are placed in 1.8 per cent urea, they swell and hemolyze exactly as they do in distilled water. Obviously, the two solutions do not have physiologically equivalent osmotic pressures. Although isosmotic, they are not isotonic. The reason for this minor dilemma is the following. Red cell membranes, like most other cell membranes, are nearly as permeable to urea as they are to water. Therefore urea exerts no osmotic effect when separated from cell contents by membranes permeable to it. A solution of urea is physiologically equivalent to distilled water.

Capillaries are relatively permeable not only to water but also

to the major crystalloids of the blood plasma. In fact they restrict only the diffusion of colloidal proteins and lipids. Hence only transient and minor osmotic forces are developed across capillary walls when solutions of higher or lower osmolal concentrations are introduced into the circulation.

An osmotic pressure is a virtual pressure rather than a real pressure. It exists only when solution and solvent or two solutions of differing osmolal concentrations are separated by a membrane permeable to solvent but not to solute. It is commonly stated that a concentrated solution has a higher osmotic pressure than a dilute solution. This statement is ambiguous for two reasons. First, as noted above, the osmotic pressure is that hydrostatic pressure which must be applied to a solution to prevent inward migration of solvent across a semipermeable membrane. No hydrostatic pressure exists in either solvent or solution in isolation. Second, the force involved is dependent on diffusion of solvent, not per se on the presence of solute except insofar as it determines the concentration of solvent in a solution.

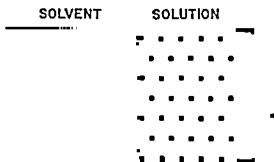


Fig 4. Kinetic formulation of osmotic pressure in terms of bidirectional diffusion of solvent across a membrane impermeable to solute.

One can formulate a kinetic picture of osmotic pressure in terms of the molecular motions of the solvent on the two sides of a cell membrane as illustrated in Figure 4. On the pure solvent side (or side of more dilute solution), water molecules in their unordered agitation strike against the membrane, and since it is permeable to water, some pass through. The same thing happens on the solution

side of the membrane with this difference; the water is somewhat diluted by the presence of the solute, so that its effective concentration, chemical potential, or escaping tendency is reduced. As a consequence, fewer water molecules per second pass from solution to solvent than in the reverse direction. The result is that there is a net flow of water from solvent to solution. The osmotic pressure is that hydrostatic pressure which must be applied to the solution to raise its chemical potential or escaping tendency to equal that of the pure solvent.

It is an unfailing source of surprise to the student when he is reminded of the osmotic forces developed when blood plasma, interstitial fluid, and cell contents are separated by semipermeable membranes from the pure solvent, water. These forces are of the order of magnitude of 6.7 atmospheres or 5,000 mm. Hg. His surprise is no doubt based on his greater familiarity with the more modest hydrostatic pressures which exist in the vascular system. The equality of osmolal concentrations of extracellular, transcellular and intracellular fluids results directly from the rapid distribution of water through capillary walls and cell membranes among all body fluid compartments, driven by what are potentially forces of great magnitude. The water distributes so that it has the same escaping tendency or chemical potential in each compartment.

The osmotic force which develops across a semipermeable membrane separating pure solvent from solution is dependent on the number of particles of solute per unit volume of solution. One gram molecular weight of glucose, namely 180 gm., containing 6.06×10^{23} particles, is termed 1.0 osmol. One osmol of glucose dissolved in 22.4 liters of water depresses the chemical potential of the water by 1.0 atmosphere; i.e., a hydrostatic pressure of this magnitude must be applied to raise the chemical potential of the water in the solution to equal that of pure water. Or conversely 1.0 osmol of glucose in 1.0 liter of water depresses its chemical potential by the equivalent of 22.4 atmospheres. The similarity between the osmotic effect of a solute in solution and the pressure it would exert if it were a gas confined in the same volume is evident. In general the laws of osmotic pressure are similar to the gas laws. One gram molecular weight of sodium chloride, namely

58.5 gm., containing 6.06×10^{23} molecules, dissociates into twice the number of ions in solution. Therefore 1.0 mol of sodium chloride exerts an osmotic effect equivalent to 2.0 osmols.[9] Calcium chloride, $CaCl_2$, dissociates into three particles; hence one gram molecular weight (111 gm.) exerts an osmotic effect of roughly 3.0 osmols.

The concentrations of the osmotically active components of the body fluids are commonly expressed in milliosmols (mOsm.) per liter or per Kg. of water; 1.0 mOsm. equalling 1/1000 osmol. One mOsm. of any solute, consisting of 6.06×10^{20} particles dissolved in a liter of water exerts an osmotic effect equivalent to 17 mm. Hg. The osmotic activity of an electrolyte solution is dependent solely on numbers of particles, not on their charge. Accordingly, 1.0 mOsm. of univalent ions, Na, K, Cl, HCO_3 consists of the same number of particles as 1.0 mOsm. of divalent ions, Ca, Mg, SO_4. One mOsm. of univalent ions is 1.0 mEq.; 1.0 mOsm. of divalent ions is 2.0 mEq. Plasma, the transcellular fluids, interstitial fluid and cellular fluids have osmolal concentrations of roughly 300 mOsm per liter of water. Therefore the osmotic effect of the solutes contained in these fluids is equivalent to $17 \times 300 = 5100$ mm. Hg. Between 90 and 95 per cent of the osmotic activity of the solutes of plasma and interstitial fluid may be assigned to sodium, chloride and bicarbonate ions. Other ions and organic compounds such as glucose, amino acids and urea account for the remaining 5 to 10 per cent.

COLLOID OSMOTIC PRESSURE AND DISTRIBUTION OF FLUID

The plasma proteins are substances of very high molecular weight· albumin, 69,000, globulins, 90,000 to over 1,000,000. Therefore, even though they are present in plasma in high concentration, 60 to 70 gm. per liter, they exert only small osmotic effects, equivalent on an average to a pressure of 28 mm. Hg. When one compares this protein or colloid osmotic effect with the total crys-

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[9]Actually less, for the activity of a sodium chloride solution of finite concentration is not 1.0, i.e., the sodium and chloride ions interact and behave as though there were slightly less than two ions per molecule.

talloidal osmotic effect of 5100 mm. Hg, it seems rather insignificant. However, it has a physiological significance out of all proportion to its quantitative magnitude. As was pointed out above, the capillary walls are permeable not only to water, but also to all those crystalloidal solutes which account for the major fraction of the osmotic effect of the plasma and interstitial fluid. Accordingly, the crystalloids exert no osmotic effect across the capillary wall. In contrast the capillaries are relatively impermeable to protein and the protein concentration of lymph from most organs other than the liver is low. As a consequence, the plasma proteins exert nearly their full osmotic effect across the capillary membrane and oppose the filtration of fluid from capillary lumen to tissue interstices under the head of hydrostatic pressure which exists in the terminal vascular bed. Because albumin is the most abundant of the plasma proteins and has the lowest molecular weight, it exerts the major fraction of the colloid osmotic effect.

TOTAL BODY STORES OF IONS

Sodium. The total body sodium of a normal adult male averages 60 mEq. per Kg. of body weight. A 70 Kg. man, therefore, contains in all 4200 mEq., or nearly 100 gm., of sodium. Bone, which constitutes only 15 per cent of body weight, contains from 40 to 45 per cent of the total sodium store, namely 1800 mEq. Of the 2400 mEq. of non-bone sodium, between 2000 and 2200 mEq. are present in the extracellular fluid. In round figures, 50 per cent of body sodium is extracellular, 40 per cent is associated with bone and 10 per cent is intracellular.

A more useful breakdown of sodium stores is into exchangeable and non-exchangeable moieties. Exchangeable sodium, measured by dilution of the radioactive isotopes Na^{22} or Na^{24}, amounts to 42 mEq. per Kg. of body weight. This fraction includes all of extracellular sodium, all of intracellular sodium and somewhat less than half of bone sodium. The non-exchangeable fraction, amounting to 18.0 mEq. per Kg. of body weight, is largely associated with bone. Exchangeable sodium is of interest in that it is in diffusion equilibrium with plasma sodium. If sodium is lost from blood plasma into urine or into feces (diarrhea), that of the labile exchangeable

reservoir is available to reduce the fall in concentration when body water is restored. If sodium is retained in the body, as it is in developing edema, it is distributed into this labile exchangeable reservoir. The ion content of this reservoir can be measured in man by the technique of isotope dilution. While scarcely a bedside procedure, it is possible to employ this method to quantify precisely the extent of sodium retention or depletion (see below for method). Non-exchangeable sodium probably represents that adsorbed on the surfaces of the apatite crystals of bone, more specifically onto surfaces which are buried in the bone structure and completely isolated from blood plasma and interstitial fluid.

Potassium. The total body potassium of a normal adult male averages 45 mEq. per Kg. of body weight. A 70 Kg. man, therefore contains in all 3150 mEq. or about 120 gm. of potassium. This potassium is almost entirely intracellular, only 60 mEq. or about 2 per cent is distributed in the extracellular fluid. Essentially all of body potassium is labile and exchangeable. As is evident from Table I, the concentration of potassium in blood plasma and interstitial fluid is low, some 4 mEq. per liter. Unfortunately, the plasma concentration of potassium is a very poor indicator of tissue potassium stores. In acute renal failure the discharge of a very small proportion of the large store of intracellular potassium will cause the extracellular concentration to rise to dangerous and possibly lethal levels of 8 to 10 mEq. per liter. On the other hand, in diabetic acidosis with normal renal function, a significant fraction of cellular potassium may be discharged into the extracellular fluid and excreted in the urine with little disturbance in plasma concentration. In chronic potassium depletion produced by prolonged vomiting or diarrhea, or by intensive diuretic therapy, sodium may replace an appreciable fraction of the potassium within cells. Plasma potassium is low under these circumstances, but rarely lower than 2 mEq. per liter. In contrast the infusion of glucose causes the transfer into cells of some 30 mEq. of potassium, which though it increases the total intracellular stores insignificantly, causes a sharp fall in plasma concentration.

All diuretics, some more than others, promote the excretion of potassium. If dietary intake is adequate, little concern need be felt

about the possibility of potassium depletion. However, it should be a matter of concern if intake is inadequate and/or diuretic therapy is strenuous and prolonged. Although it is possible to measure total body stores of potassium by isotope dilution, in clinical practice it is rarely feasible. One must usually resort to a therapeutic test of the response to potassium administration in those instances in which potassium depletion is suspected.

Chloride. The total body chloride of a normal adult male averages 33 mEq. per Kg. of body weight. A 70 Kg. man, therefore, contains in all 2310 mEq. or about 82 gms. of chloride. The major part of this chloride (about 70 per cent) is distributed in the plasma and interstitial fluid. It can therefore be considered as being primarily an extracellular ion, but not, as has so frequently been claimed, an exclusively extracellular one. The 30 per cent of the chloride beyond the confines of the extracellular compartment is in part intracellular and in part localized in connective tissue. Of all cells, the erythrocyte contains the most chloride. Cells of the testis, ovary, gastric mucosa and skin contain lesser amounts. That present in skin may well be localized mainly in the connective tissue of the dermis. In fact collagenous fibers, wherever located, seem to be relatively rich in chloride. This has led to the description of a separate extracellular phase termed the connective tissue compartment. A reasonable view is that chloride ions are adsorbed on collagen fibers much as sodium ions are adsorbed on the apatite crystals of bone. This chloride is on the surface of the fibers, and is exchangeable.

Bicarbonate. The bicarbonate ion is unique among the electrolytes of the body fluids. It has no permanence as an ion species. Its existence is fleeting and is merely a step in the transfer of metabolic carbon dioxide from tissues to lungs. In fact one can best look upon bicarbonate as an anion which represents the excess of cations such as sodium, potassium, calcium and magnesium, over fixed anions such as chloride, phosphate, sulfate and protein. Since carbon dioxide and water are ubiquitous within the body and since cationic charges must be exactly neutralized by anionic charges, any cation excess (imposed by the liberation of alkali within the body) is immediately balanced by the hydration of carbon dioxide to form

bicarbonate ions. Conversely any anion excess (imposed by the liberation of acid within the body) is immediately balanced by the dehydration of bicarbonate to form carbon dioxide and water.

The total body content of bicarbonate averages 10 to 12 mEq. per Kg. About half of the total is distributed in the extracellular compartment, the remainder in tissues. The CO_2 in bone is largely in the form of carbonate, bound in the lattice, occluded, and largely non-exchangeable. Presumably all bicarbonate of cells is exchangeable. It is obvious that the concentration of bicarbonate in cells is only a fraction of that in extracellular fluid, for about one third of the total body store is distributed through two thirds of total body water. However, cell concentrations vary in different tissues and are known with no certainty.

MEASUREMENT OF BODY STORES OF IONS

The total ionic content of the human body has been measured in only a few instances by complete dissolution of the cadaver and analysis of aliquots of the resulting solution. A method more appropriate to the study of normal subjects and of the alterations produced by disease is the isotope dilution method for measuring the exchangeable ion content of the body. The exchangeable ion content is less than the total ion content by the quantities of ions occluded in the crystal lattice of the bone and in minor degree, separated from plasma by the relatively impermeable blood-brain barrier.

The method for determining the exchangeable ion content of the body may be illustrated for sodium. Radioactive sodium-22 or 24 distributes throughout the body exactly as does non-radioactive sodium, except for the two reservoirs of occluded sodium noted above. If one injects some 20 to 40 microcuries of radiosodium intravenously, allows 24 hours for it to equilibrate with sodium stores of the body, withdraws a blood sample and analyzes the plasma for both radiosodium (in terms of counts per min. per ml.) and non-radiosodium (in terms of mEq. per ml.), it is possible to calculate the exchangeable sodium content of the body according to the following equation:

$$\text{Exchangeable Na (m.Eq)} = \frac{\text{counts/min. given} - \text{counts/min. excreted}}{\dfrac{\text{counts/min./ml. plasma}}{\text{mEq. Na/ml. plasma}}}$$

All urine formed during the 24 hour equilibration period is collected and an aliquot is counted to determine the quantity of radiosodium excreted. The numerator of the equation given above is the quantity of radiosodium present in the body at the time of drawing the blood sample. The denominator is the specific activity of sodium in plasma, i.e., counts/min./mEq. of sodium.

If 45,000,000 counts per min. are given as radiosodium and if 5,000,000 counts per min. are excreted in 24 hrs., there remain in the body 40,000,000 counts per min. The plasma radioactivity at this time might be 2000 counts per min. per ml. and the sodium content might be 0.140 mEq. per ml. (140 mEq. per liter). Substituting in the equation above gives:

$$\text{Exchangeable Na (mEq.)} = \frac{45{,}000{,}000 - 5{,}000{,}000}{2000/0.140} = \frac{40{,}000{,}000}{14{,}285} = 2{,}800 \text{ mEq.}$$

If the individual weighs 70 Kg., total exchangeable sodium amounts to 40 mEq. per Kg. of body weight. This method has been utilized to determine the exchangeable sodium, potassium, chloride and bicarbonate ion contents of the body.

SUMMARY

Sodium is the major cation and chloride and bicarbonate are the major anions of plasma and interstitial fluid. These ions represent some 90 to 95 per cent of the osmotically active components of extracellular fluid. The capillary endothelium is relatively permeable to cations and to anions other than protein. Ions and water, therefore, distribute rapidly between plasma and interstitial fluid. Total osmolal concentrations and compositions of the two phases are nearly equal, differing slightly because of the presence of non-diffusible protein anions in plasma.

Potassium and magnesium are the major cations and protein and organic phosphate complexes are the major anions of intracellular fluid. Cell membranes are effectively impermeable to both anions and cations. Impermeability to protein and organic phosphate complexes is absolute. Impermeability to sodium and potassium is

relative and dependent on the continuous operation of ion pumps which extrude sodium from the cell and concentrate potassium within the cell.

Since water diffuses rapidly across cell membranes, the osmolality of intracellular fluid is essentially the same as that of extracellular fluid. Sodium diffuses into cells but is pumped out to maintain the intracellular concentration low. Potassium diffuses out of cells but is pumped in to maintain intracellular concentration high. Cellular sodium and potassium ions therefore exchange readily with their extracellular counterparts. A part of body sodium is adsorbed on the crystal lattice of bone, buried deeply in its structure, and therefore non-exchangeable. Essentially all of body potassium is exchangeable.

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$$\text{Exchangeable Na (m.Eq.)} = \frac{\text{counts/min. given} - \text{counts/min. excreted}}{\dfrac{\text{counts/min./ml. plasma}}{\text{mEq. Na/ml. plasma}}}$$

All urine formed during the 24 hour equilibration period is collected and an aliquot is counted to determine the quantity of radiosodium excreted. The numerator of the equation given above is the quantity of radiosodium present in the body at the time of drawing the blood sample. The denominator is the specific activity of sodium in plasma, i.e., counts/min./mEq. of sodium.

If 45,000,000 counts per min. are given as radiosodium and if 5,000,000 counts per min. are excreted in 24 hrs., there remain in the body 40,000,000 counts per min. The plasma radioactivity at this time might be 2000 counts per min. per ml. and the sodium content might be 0.140 mEq. per ml. (140 mEq. per liter). Substituting in the equation above gives:

$$\text{Exchangeable Na (mEq.)} = \frac{45{,}000{,}000 - 5{,}000{,}000}{2000/0.140} = \frac{40{,}000{,}000}{14{,}285} = 2{,}800 \text{ mEq.}$$

If the individual weighs 70 Kg., total exchangeable sodium amounts to 40 mEq. per Kg. of body weight. This method has been utilized to determine the exchangeable sodium, potassium, chloride and bicarbonate ion contents of the body.

SUMMARY

Sodium is the major cation and chloride and bicarbonate are the major anions of plasma and interstitial fluid. These ions represent some 90 to 95 per cent of the osmotically active components of extracellular fluid. The capillary endothelium is relatively permeable to cations and to anions other than protein. Ions and water, therefore, distribute rapidly between plasma and interstitial fluid. Total osmolal concentrations and compositions of the two phases are nearly equal, differing slightly because of the presence of nondiffusible protein anions in plasma.

Potassium and magnesium are the major cations and protein and organic phosphate complexes are the major anions of intracellular fluid. Cell membranes are effectively impermeable to both anions and cations. Impermeability to protein and organic phosphate complexes is absolute. Impermeability to sodium and potassium is

plasma is 6 to 7 per cent, whereas that in interstitial fluid is 1 per cent or less. Since the capillary endothelium is more or less impermeable to proteins, they exert a colloid osmotic (oncotic) effect equivalent to a pressure of 25 mm. Hg, 25 mm. Hg so oriented as to resist filtration from the vascular compartment. Since the capillary endothelium is permeable to crystalloidal solutes, they exert no osmotic effects on fluid distribution across the vessel wall.

A third force, the tissue turgor pressure, which amounts to 2 to 5 or more mm. Hg., can also be considered as one resisting the outward filtration of fluid. The sum of these forces is equivalent to a net outward or filtration pressure of 10 to 15 mm. Hg.

Energy is expended in driving blood through the capillaries, for length is far greater in relation to diameter than is shown in the diagram. Therefore, hydrostatic pressure drops to some 10 to 15 mm. Hg. at the venular end. The oncotic effect and the tissue turgor pressure, both of which resist filtration, are essentially the same at the two ends of the capillary. Therefore, at the venular end the sum of these forces is equivalent to a net inward or absorbing pressure of 10 to 15 mm. Hg. According to the Starling hypothesis, outward filtration of fluid at the arteriolar end and reabsorption at the venular end of the capillary causes a slow circulation of fluid through the tissue interstices as illustrated in Figure 5.

Diffusion vs. Filtration and Reabsorption in Tissue Nutrition. The view that tissue nutrition is largely dependent on this circulation of interstitial fluid is one which is commonly held. It is completely erroneous. It is evident from the work of Landis, Pappenheimer and others that the outward filtration of fluid at the arteriolar end of the capillary and the inward flow at the venular end are in reality quite small. Pappenheimer has calculated that pressure differences of the order of those shown in Figure 5 would cause the filtration and reabsorption of only 0.003 ml. of fluid per min. across all of the capillaries contained in 100 gm. of tissue in the human forearm, i.e., a total volume of only 40 ml. in 24 hr. It is evident from these estimates that the transport of substances to and from tissues would be extremely slow and entirely inadequate to serve their metabolic needs, if the mechanism of transport were limited

Chapter III

VASCULAR AND INTERSTITIAL FLUID EXCHANGES; EDEMA

The Starling Hypothesis. The factors and forces determining the distribution of fluid between vascular and interstitial compartments were first clearly outlined by Starling in 1896. The elements of his thesis are represented in highly schematic form in Figure 5. There exists within the arteriolar end of the capillary a hydrostatic pressure of 40 to 45 mm. Hg. This pressure is less than aortic pressure, due to the dissipation of energy in overcoming resistance to flow in small arteries and arterioles. It represents the residual force available to drive the blood onward through capillaries,

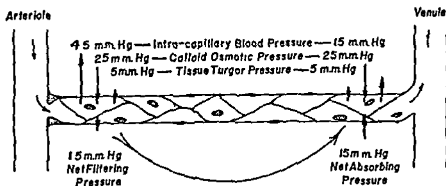


Fig. 5.

venules, and veins to the heart. It is also in part available to drive fluid outward through the porous endothelial walls of the capillaries into the interstitial spaces. However, the entire force is not available for this later purpose, for the concentration of proteins in

veins. True capillaries arise from and rejoin the preferential channels. Smooth muscle fibers at the points of origin of true capillaries serve as sphincters to control the perfusion of the capillary loops. When the sphincters relax, brisk perfusion of the loops occurs. When the sphincters contract, flow through the loops ceases.

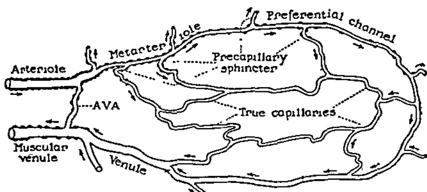


Fig. 6. Organization of the terminal vascular bed. (From B. W. Zweifach: *Tr. 3rd. Jonah Macy, Jr Conference on Factors Regulating Blood Pressure. 1949.)*

Rhythmic contraction and relaxation of precapillary spincters is observed under normal conditions and is termed vasomotion. Frequency of vasomotion and relative duration of constrictor and dilator phases together determine the minute volume of blood which perfuses a tissue and are dependent on activity of vasomotor nerves, metabolites formed in the tissues, and hormones carried in the blood stream. Zweifach has shown that during active blood perfusion, fluid filters out into the interstitium, not only at the arteriolar end but throughout the entire length of the capillary loop. During the constrictor phase, perfusion ceases, pressure drops and fluid returns to the capillary lumen throughout its length. Thus prolongation of the constrictor phase favors reabsorption of fluid, prolongation of the dilator phase favors filtration of fluid.

Lymphatic Drainage of Interstitial Fluid. The lymphatic system consists of a meshwork of delicate lymph capillaries ramifying through the tissue interstices. These capillaries begin in the periphery as blind endothelial tubes which progressively coalesce in their central course to form thicker walled lymphatic channels.

to bulk flow through the filtering and absorbing regions of the capillaries.

Diffusion of water and solutes in both directions across the capillary wall occurs at phenomenally high rates in comparision with the rates of transport of these substances by bulk filtration and reabsorption. Pappenheimer has calculated that the plasma contained in the capillaries of the human forearm exchanges its water with that of the surrounding interstitial fluid some 300 times per min. The sodium chloride, urea, and glucose of capillary plasma are exchanged 120, 100, and 40 times per min., respectively, with that of interstitial fluid. These rates of course describe exchange, not net transfer. However, if a tissue utilizes glucose and lowers concentration slightly in the interstitial fluid, net diffusion of glucose from plasma to tissue will occur. Similarly, any metabolite produced in the tissue will diffuse in the opposite direction when the concentration in interstitial fluid increases slightly above that in plasma.

High rates of exchange and adequate net transfer across capillary walls between blood plasma and tissues depend on the very short path over which diffusion takes place, not on extreme porosity of the endothelial membrane. According to Pappenheimer, the individual pores of muscle capillaries through which diffusion occurs have apparent diameters of 65 Å (1 Å = 1/10,000,000 mm.), and a population density of 10^9 per cm^2. However, pore orfices make up only 0.1 per cent of the endothelial surface, 99.9 per cent of the surface is impermeable to water and solutes. Rapid diffusion depends on the fact that the pores are only 600 Å in length (thickness of the capillary endothelium).

Role of Vasomotion in Filtration and Reabsorption of Interstitial Fluid. The concept of filtration of fluid at the arteriolar end of a capillary and of reabsorption at the venular end, as illustrated in Figure 5, is no doubt a gross over-simplification. Zweifach has shown that the functional organization of the terminal vascular bed can be more adequately described in terms of the diagram shown in Figure 6. Arterioles and metarterioles lose their envestments of smooth muscle, continue directly as endothelial loops termed preferential channels, and ultimately become venules and

hold the capillary open against a minute hydrostatic pressure gradient across the capillary wall. On the other hand, pinocytosis (cell drinking of fluid) and phagocytosis observed in a variety of cells in tissue culture, might account for transfer of fluid and particulate matter into the lumen of the lymph capillaries. Suffice to say that, although the forces at play are unknown, interstitial fluid enters terminal lymphatics and progresses centrally to be discharged into the superior caval venous reservoir. Centripetal flow is assisted by muscular activity in the extremities, by abdominal tone, and by the suction pump of the thoracic bellows, and is directed by a series of valves scattered along the lymphatic channels. Pulsatile lymph hearts, especially prominent in amphibia, are absent in mammals.

Rate of Lymph Flow. The flow of lymph under normal conditions and especially at rest is quite small in the mammal. The flow in man, measured in individuals with fistulae of the thoracic duct, varies from 1 to 2 ml. per min. Judging from results on dogs, well over half of total lymph flow is derived from the liver and gut. It is clear that if any appreciable volume of fluid is filtered through capillary loops during the dilator phase of vasomotion, it must be rather completely reabsorbed into the same or other loops during the constrictor phase. Obviously the lymphatic system is relatively unimportant for the removal of fluid from tissues of mammals. However, continued leakage of protein and reabsorption of protein free fluid would soon build up the colloid concentration of interstitial fluid to levels which would interfere with the normal oncotic effects operating to retain fluid in the vascular system.

FACTORS FAVORING EXPANSION OF INTERSTITIAL FLUID VOLUME

Prolongation of the Dilator Phase of Vasomotion at the expense of the constrictor phase leads to the collection of excessive amounts of interstitial fluid in the tissues, for as pointed out by Zweifach, fluid filters through the walls of actively perfused capillaries along their entire length, not solely at their arteriolar ends as postulated by Starling. Hyperemia is characterized by prolongation of the dilator phase of vasomotion relative to the constrictor phase. Therefore hyperemia, whether its cause be inflam-

Channels from the lower extremities combine in the abdomen with those from the viscera to form the cisterna chyli, a multilocular plexus of vessels in the celiac region. Lymph from the cisterna chyli flows into the thoracic duct, a well defined vessel lying to the left of the midline posteriorly, to enter the venous system at the junction of the left subclavian and jugular veins. Lymph from the left head and neck, from the left upper extremity, and from thoracic structures on the left flows into the thoracic duct before it enters the venous system. A similar but less extensive system draining the right thorax, right upper extremity and right side of the head and neck drains into the right subclavian vein. Scattered along the smaller tributaries are lymph glands which subserve the several functions of mechanical removel of particulate matter, formation of lymphocytes and production of antibodies.

As has been pointed out previously, the protein concentration of lymph derived from skin and muscle is low, that from gut is intermediate, and that from liver is high. Differences in concentration are obviously an expression of differences in permeability of the capillaries of these several regions to protein. The concentration of protein is always higher in plasma than in interstitial fluid. Short of active secretion, there is no way to return protein to the vascular compartment other than by a system of drainage canals. A major function of the lymphatic system appears to be the return of protein from the interstitial to the vascular compartment.

The thin walled endothelial tubes making up the terminal ramifications of the lymphatic system are evidently much more permeable to proteins, colloidal dyes and even particulate matter than are the blood capillaries, for these materials are readily picked up and removed from the interstitial spaces by the lymphatics. The nature of the forces which operate to cause the entry of colloid-containing fluid and especially particulate matter into the lymph channels is unknown. One finds it difficult to explain such entry entirely on the basis of hydrostatic (tissue turgor) pressure, for such a pressure would be exerted equally inside and outside the lymph capillary in a fluid filled system. However, the endothelial walls of the lymph capillaries are attached to neighboring tissue cells and collagen fibers by fine protoplasmic strands. Perhaps these

Increase in Capillary Permeability with loss of protein into tissue interstices reduces the differential oncotic effects which ordinarily restrain fluid in the vascular compartment; i.e., the protein concentration of the interstitial fluid approaches that of the plasma. A number of factors account for the relative impermeability of normal capillaries. Protein (possibly fibrinogen, adsorbed in a mono-molecular film on the inner surface of the capillary, leak-proofs it. The endothelials cells are locked together in a tongue and groove arrangement and the chinks are filled with calcium proteinate cement impermeable to colloids. The endothelial cells themselves possess tone or turgesence which enables them to withstand the hydrostatic force distending the capillary. If tone is reduced, or conversely if compliance is increased, the capillary loses its normal impermeability to protein. Physical damage by heat or ultraviolet light, capillary poisons, increased acidity of blood, anoxia, lack of calcium, and the liberation of proteolytic enzymes and histamine in damaged tissues all increase capillary permeability to protein and thus increase transudation of fluid.

Increased Tissue Distensibility, i. e., loss of tissue elasticity in the aged or in those who have suffered weight loss favors expansion of extracellular fluid volume. Stretching of subcutaneous tissues during a previous episode of edema predisposes to subsequent bouts. Laxness of connective tissue around the eyes favors the development of suborbital and periorbital edema. Tissue turgor is one of the forces in the Starling hypothesis which resists outward filtration in actively perfused capillaries and which favors reabsorption in those minimally perfused.

Obstruction of Lymphatic Drainage of a degree sufficient to produce manifest and persistent edema is almost always the result of long standing chronic or repeated acute inflammatory processes. Lymph edema is of a brawny type, characterized by marked connective tissue proliferation and by the collection of proteinaceous fluid in the tissue interstices. It is seen in classical form in elephantiasis due to filarial infestation. In lesser degree it is seen following repeated attacks of erysipelas. Lymphatics have a very great capacity for regeneration and only repeated insults result in permanent lymphatic blockage and lymph edema.

mation, the local injection of histamine or the local application of heat, is accompanied by a local increase in formation of interstitial fluid. Three of the cardinal signs of inflammation, *rubor* (redness) and *calor* (heat), both related to increased blood flow, and *turgor* (increased interstitial fluid volume and pressure) are causally related.

Increase in Intracapillary Hydrostatic Pressure increases the net filtering force which causes transudation of fluid into tissues. Arteriolar dilation results in an increase in filtration pressure. Less of the potential energy imparted by the beat of the heart is dissipated in overcoming frictional resistance to flow in the arterioles; more is available to filter fluid through capillary walls. An increase in filtration pressure likewise results from an increase in venous pressure. If venous outflow is obstructed, pressure rises in the capillary bed and transudation of fluid increases. The rise in venous pressure may be local, affecting a single extremity, e.g., thrombophlebitis of the femoral vein, or it may be general and affect all capillary beds, e.g., chronic congestive heart failure. However, in congestive failure edema first collects in dependent parts of the body in which, as a result of gravity, the intracapillary hydrostatic pressure is greatest.

Reduction in Concentration of Serum Proteins reduces the colloid osmotic effect which operates to hold fluid within the vascular compartment. In the classical interpretation of Starling, low serum protein concentration favors excessive filtration at the arteriolar end of the capillary and reduced reabsorption at the venular end. According to Zweifach, it favors excessive filtration in actively perfused capillaries and reduced reabsorption in those which are not perfused. Low serum protein concentration is characteristic of severe protein starvation, chronic liver disease with cirrhosis and the nephrotic syndrome. In the latter condition, low serum proteins result from the massive loss of albumin in the urine. The normal 2 : 1 ratio of albumin/globulin in the serum is reduced and even reversed. Since the oncotic effect of the serum proteins is largely dependent on the albumin concentration, it suffers a larger decrement in the nephrotic syndrome than might be anticipated from the degree of reduction in total protein concentration.

left of Table III. In failure of the heart as a pump, cardiac output decreases and the heart fails to empty itself. As a consequence, central venous filling pressure rises and the increase in pressure is reflected back into peripheral veins, venules and capillaries. Increased transudation of fluid through capillary walls leads to the collection of edema and to reduction in effective circulating plasma volume. The kidneys retain salt and water in isotonic proportions and fluid is diverted to the periphery. Some have inferred that passive venous congestion and stagnant anoxia of the kidneys in congestive heart failure reduce their capacity to excrete salt and water. However, the major emphasis has been on increased capillary transudation and prerenal diversion of fluid.

The Edemas of Nephrosis, Cirrhosis and Nephritis were early explained along similar lines. In the nephrotic syndrome, massive loss of albumin in the urine was thought to lead to hypoproteinemia, reduced plasma oncotic pressure, increased transudation, edema and prerenal diversion of fluid in causal sequence. The edemas of severe protein starvation and of cirrhosis were likewise considered to be due at least in part to hypoproteinemia. However, in cirrhosis the factor of increased portal pressure was recognized as of major significance. In nephritis, emphasis was placed on a vasculitis affecting not only the terminal vascular bed of the kidneys but that of other viscera and of systemic structures as well. This vasculitis, increasing capillary permeability to protein, resulted in a reduction in the oncotic effect which holds fluid within the vascular compartment. Again the significant sequence was increased transudation, edema and prerenal diversion of fluid.

MODERN CONCEPTS OF THE PATHOGENESIS OF EDEMA

Present concepts of the pathogenesis of edema differ from those just presented more in emphasis than substance. The major differences relate to the role of the kidneys in the retention of salt and water and to the sequence of events leading to the formation of edema.

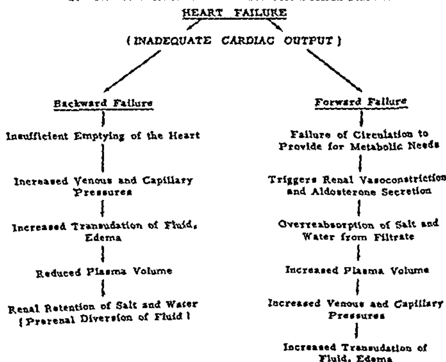
Role of the Kidneys in the Pathogenesis of Edema. The concept that prerenal diversion of fluid into tissue interstices accounts for renal retention is a semantic simplification without justification of fact. In even the most severely edematous patient, the amount

EARLY CONCEPTS OF THE PATHOGENESIS OF EDEMA

For the first four decades of this century the view was commonly held that generalized edema, whatever its cause, could be more or less completely explained in terms of Starling's hypothesis. It was maintained that one or more of the forces favoring capillary transudation, namely increased hydrostatic pressure, reduced plasma oncotic pressure or increased capillary permeability is primarily increased in edematous patients. Salt and water are retained in the body because that which is absorbed by the gut is diverted into the tissues as edema, little is presented the kidneys for excretion. A necessary corrolary of increased transudation and prerenal diversion of fluid as primary causes of edema is relative anhydremia and oligemia. By no means are these universal findings.

Backward Failure of the Heart. The general features of the pathogenesis of edema outlined above may be illustrated in terms of the concept of backward failure of the heart, summarized on the

TABLE III
THE PATHOGENESIS OF EDEMA IN CONGESTIVE HEART FAILURE



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Role of the Kidneys in the Pathogenesis of Edema. The concept that prerenal diversion of fluid into tissue interstices accounts for renal retention is a semantic simplification without justification of fact. In even the most severely edematous patient, the amount

of salt and water presented to the kidneys in the renal arterial blood is many orders of magnitude greater than that which would have to be excreted to achieve fluid balance. The normal individual achieves fluid balance by excreting each day a fraction of one per cent of the salt and water delivered into the renal tubules in the glomerular filtrate. More than 99 per cent of the quantities filtered is reabsorbed. Although the severely edematous patient may form less glomerular filtrate each day, balance could be achieved by the excretion of a very few per cent of the filtered quantities. Instead little salt and less than normal quantities of water are excreted during the phase of accumulation of edema. It is evident that absolute or relative over-reabsorption of salt and water from the glomerular filtrate, not prerenal diversion, ultimately accounts for renal retention.

It is now apparent that two alterations in renal function in varying degree underlie the failure of edematous patients to excrete salt and water. Certain patients have low rates of glomerular filtration. Less than normal quantities of sodium and water are delivered into the renal tubules each day, yet tubular reabsorption continues at nearly the usual rate. As a consequence, all is reaborbed; none is excreted. Other edematous patients have normal rates of glomerular filtration. Tubular reabsorption of salt and water is enhanced, in fair part due to stimulation by excessive production of adrenal salt retaining hormones; as a consequence, excretion is reduced. These two factors, reduced filtration and hormonally stimulated over-reabsorption, appear to be synergistic causes of *retention of salt and water in congestive heart failure, cirrhosis, the* nephrotic syndrome, acute nephritis, and eclampsia, and no doubt are operative in other forms of generalized edema as well. However, the relative roles of these two factors differ from patient to patient and from time to time in a given patient. The nature of these alterations in renal function will be considered in greater detail in Chapter VI.

If renal retention of salt and water is of primary significance, one would expect to observe plethora and hydremia in edematous patients; both are common findings. A part of the retained fluid filters out into the tissues in accordance with the balance of forces

outlined in Starling's hypothesis. It is distributed first to sites of highest hydrostatic pressure and lowest tissue turgor pressure. Rate of accumulation is enhanced by hypoproteinemia and by increased capillary permeability. While his successors placed major emphasis on the balance of forces across capillary walls in explaining the pathogenesis of edema, as long ago as 1896 Starling suggested the possibility that diminished excretion of fluid by the kidneys could result in hydremia and that hydremia might be the cause of increased effective capillary filtration pressure and edema.

Forward Failure of the Heart. The general features of the pathogenesis of edema outlined above may be illustrated in terms of the concept of forward failure of the heart, summarized on the right of Table III. As a consequence either of intrinsic disease of the heart, which reduces its work capacity, or of extrinsic demands, which exceed its work tolerance, the cardiac output becomes inadequate to satisfy the metabolic demands of the body. Thus failure may develop in the presence of reduced cardiac output (valvular heart disease, acute or chronic myocarditis; arteriosclerotic heart disease) or of increased cardiac output (thyrotoxicosis, beriberi, severe anemia, massive arterio-venous fistula). One must postulate that failure of the circulation to provide for metabolic needs triggers mechanisms for salt and water conservation. These mechanisms include renal vasoconstriction, which reduces renal blood flow and glomerular filtration rate, and stimulation of secretion of adrenal steroids, which promote renal tubular reabsorption of salt. Salt and water are retained in the vascular compartment inducing hydremia and plethora. Venous and effective capillary filtration pressures rise. As a consequence of increased transudation, interstitial fluid volume increases.

The weak element of the argument is that as yet no mechanism has been satisfactorily described which senses adequacy of cardiac output relative to metabolic demands and which translates that information into the neural and hormonal control of tubular reabsorption of salt and water. However, the fact that such a mechanism has not been characterized does not deny its existence. In favor of the hypothesis are the following facts. Starr has shown in patients dying in congestive failure that the adynamic filling pres-

of salt and water presented to the kidneys in the renal arterial blood is many orders of magnitude greater than that which would have to be excreted to achieve fluid balance. The normal individual achieves fluid balance by excreting each day a fraction of one per cent of the salt and water delivered into the renal tubules in the glomerular filtrate. More than 99 per cent of the quantities filtered is reabsorbed. Although the severely edematous patient may form less glomerular filtrate each day, balance could be achieved by the excretion of a very few per cent of the filtered quantities. Instead little salt and less than normal quantities of water are excreted during the phase of accumulation of edema. It is evident that absolute or relative over-reabsorption of salt and water from the glomerular filtrate, not prerenal diversion, ultimately accounts for renal retention.

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prolongation of the dilator phase of vasomotion, by an increase in capillary permeability, by a reduction in serum colloids, by increased tissue distensibility and by obstruction of lymphatic drainage. Ultimately the extent of edema formation is determined by the avidity with which the kidneys retain ingested salt and water.

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sure of the circulatory system is increased, i.e., the mean pressure in the vascular bed after the heart has stopped beating is elevated. This implies that the circulatory system is overfilled during life. Most studies on living patients utilizing dilution methods described earlier, have shown that circulating blood volume is increased. Furthermore, in patients developing edema, renal retention of salt and water frequently precedes elevation of venous pressure. Primacy of renal retention of fluid, stimulated by relative inadequacy of cardiac output, has the virtue of explaining edema in those conditions in which high output failure exists, e.g., thyrotoxicosis, beriberi, severe anemias and massive arteriovenous fistulae, as well as in those with low output failure.

SUMMARY

Fluid is distributed between the vascular system and the interstitial compartment in accordance with the balance of forces across the capillary endothelium. The hydrostatic pressure of the blood within the capillaries is the force responsible for the outward filtration of fluid. This is balanced by the colloid osmotic effect of the plasma proteins and by the tissue turgor pressure which tend to hold fluid in the peripheral vascular bed. According to the classical theory of Starling, fluid leaves the blood stream at the arteriolar end of the capillaries, where high hydrostatic pressure favors outward filtration, and reenters at the venular end, where low hydrostatic pressure favors absorption. Zweifach has modified this concept by showing that fluid filters outward along the entire length of the capillary during active perfusion of tissues with blood and is absorbed along the entire length during the quiescent period. Alternate perfusion and quiescence of circulation is brought about by vasomotion of the terminal vascular bed. Relatively little fluid is returned to the vascular compartment by the lymphatics. However, the lymphatics play an important role in that they permit the return to the blood of the protein which leaks through the capillary walls.

Transudation of fluid and formation of edema is favored by an increase in capillary hydrostatic pressure due either to arteriolar dilation or to the obstruction of venous return. It is favored by

prolongation of the dilator phase of vasomotion, by an increase in capillary permeability, by a reduction in serum colloids, by increased tissue distensibility and by obstruction of lymphatic drainage. Ultimately the extent of edema formation is determined by the avidity with which the kidneys retain ingested salt and water.

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Chapter IV

MECHANISMS OF RENAL FILTRATION, REABSORPTION, AND EXCRETION OF IONS AND WATER

THE kidneys regulate the volume, osmolality, and ionic composition of the extracellular fluid. If water or any of the major extracellular ions, sodium, chloride, and bicarbonate is present in the body in excess, it is eliminated in the urine; if the body store is deficient, excretion is curtailed. Urine formation begins with the ultrafiltration of large volumes of plasma through the glomerular capillary tufts. To prevent rapid exhaustion of body stores, the bulk of the filtered ions and water must be reabsorbed from the tubular urine. When intake is within the usual range, only a small fraction, less than one per cent, of the filtered sodium, chloride, bicarbonate, and water need be excreted to achieve balance. If less than normal quantities are reabsorbed, body reserves are progressively depleted and dehydration results. If reabsorption is excessive, body reserves progressively expand and edema develops. As a prelude to a description of the abnormalities of volume regulation and of renal function in edema, we shall consider in this chapter the normal renal processes of glomerular filtration, tubular reabsorption, and excretion of ions and water.

GLOMERULAR ULTRAFILTRATION OF IONS AND WATER

Simple filtration removes particulate matter from plasma; glomerular ultrafiltration carries the process a step further and removes colloids (proteins and lipids). However, an ultrafiltrate like a simple filtrate contains all crystalloidal solutes in essentially the same concentrations as exist in the aqueous phase of the original fluid. True, since the filtrand (plasma) contains protein anions while the ultrafiltrate contains none, the concentrations of crystalloidal anions (chloride and bicarbonate) will be slightly higher in

the filtrate than in the aqueous phase of plasma. Conversely the concentrations of crystalloidal cations (sodium and potassium) will be slightly lower in the filtrate than in the aqueous phase of plasma.[10] These differences in concentration, which are relatively small, are manifestations of the Gibbs-Donnan equilibrium described earlier on page 14. Uncharged crystalloids like glucose and urea have identical concentrations in the ultrafiltrate and in the aqueous phase of plasma.

Criteria of Ultrafiltration are three: (1) the ultrafiltrate must be protein free; (2) it must contain all crystalloids in exactly the same concentrations as in the aqueous phase of the plasma, except for the slight deviations demanded by the Gibbs-Donnan rule; and (3) the hydrostatic force must be adequate to account for the volume of ultrafiltrate formed per unit time. The general acceptance of glomerular ultrafiltration as the initial process in urine formation is based on the admirably direct and precise studies of Dr. A. N. Richards and his colleagues on the amphibian and mammalian kidney. Richards devised the method of puncturing Bowman's capsule of a glomerulus with a micro glass pipette, sealing off the neck of the tubule with a glass rod to prevent reflux, and collecting the filtrate by gentle aspiration as it forms.

Wearn and Richards first noted that glomerular filtrate collected in this manner from the amphibian kidney is protein-free, an observation extended to the mammalian kidney[11] by Walker, Bott,

[10]As a rough approximation, the concentrations of chloride and bicarbonate in an ultrafiltrate of normal plasma are calculated as 1.05/0.94 times the concentrations in plasma; the concentrations of sodium and potassium in an ultrafiltrate are calculated as 0.95/0.94 times the concentrations in plasma. The divisor, 0.94, corrects each expression for the water content of plasma, i.e., the ions in 1 ml. of plasma are dissolved in 0.94 ml. of water.

The dividends, 0.95 and 1.05, are the Donnan factors for univalent cations and anions, respectively, applied when ion concentrations are expressed in mEq. per unit volume of water. Both the Donnan factors and the water correction are based on a plasma protein concentration of 6 per cent and a normal plasma pH.

[11]Actually the filtrate contains no protein by the test method employed which had a lower limit of sensitivity of 30 mg per cent. The protein concentration of mammalian plasma is some 6000 mg. per cent. Since the filtrate contains less than 0.5 per cent of the protein content of plasma, it is reasonable to say that it is protein free. Actually trace amounts of protein probably are filtered and absorbed by the renal tubules. Certain mammals, including the rat, normally filter and excrete significant amounts of protein.

Oliver and MacDowell. This satisfies the initial criterion of glomerular ultrafiltration, i.e., the filtrate must be protein-free.

Richards and his colleagues then devised a series of microchemical methods applicable to the microliter or so of fluid which could be collected from a single glomerulus. They clearly demonstrated that the concentrations of glucose, chloride, sodium, urea, phosphate, uric acid and creatinine are the same in the filtrate as in the aqueous phase of the plasma. They likewise observed identity of pH, electrical conductivity and osmolal concentration. These observations satisfy the second criterion of glomerular ultrafiltration, namely that the concentrations of crystalloids are the same in plasma and filtrate.

The third criterion, i.e., adequacy of hydrostatic force, although satisfied by experimental measurements in the amphibian kidney, *must at present be accepted more on probability than rigorous* proof in the mammalian kidney. In man, with a mean arterial pressure of 100 mm. Hg. mean glomerular capillary pressure has been estimated to be 75 mm. Hg. This high value derives from the *short and direct arterial supply to the glomeruli*, the afferent arterioles arising directly from the interlobular arteries. The colloid osmotic effect of plasma proteins, equivalent to a pressure of 25 to 30 mm. Hg, and the intrarenal turgor pressure of 15 mm. Hg oppose the intracapillary hydrostatic pressure. The balance of forces yields a net filtration pressure of 35 to 40 mm. Hg. This pressure is available to overcome frictional resistance in forcing fluid through the minute pores of the glomerular capillary membrane and along the tubule from glomerulus to pelvis. According to calculations of Pappenheimer, the forces are adequate. If each glomerulus produced only one drop of filtrate in a day's time, the total filtrate formed in the 2.5 million glomeruli of two normal human kidneys would add up to the 150 to 200 liters now known to be formed in 24 hours.

Properties of the Glomerular Capillary Filter. Pappenheimer has developed an operational concept of the structure of the glomerular capillary membranes, based on his studies of their relative permeability to water and to a series of substances of varying molecular dimensions. They behave as though they were per-

forated by myriads of aqueous channels, cylindrical in form, and some 75 Å in diameter by 600 Å in length. Glomerular capillaries are far more permeable to water and solutes than are muscle capillaries. Pappenheimer ascribes this to the fact that the aqueous pores occupy 5 per cent of the surface of glomerular capillaries, only 0.1 per cent of the surface of muscle capillaries. The glomerular pores are also slightly larger. The total glomerular capillary surface of man has been estimated to be 8,000 to 16,000 cm.^{2}; the total pore area is therefore 400 to 800 cm.^{2}.

The diameter of the pores is such that they restrain absolutely serum globulin, but not free hemoglobin nor albumin. Yet little hemoglobin and much less albumin (only traces) enter the filtrate. Pappenheimer explains the sieving of these molecules, of a diameter approaching that of the pores, on the basis of probability. It is unlikely that a hemoglobin molecule, with an effective diameter[12] of 64 Å, will hit a pore of 75 Å sufficiently "head on" to enter. A few do. The likelihood that albumin, with an effective diameter of 70 Å, will do so is even less. Very few do. A second factor restricting passage of molecules into the filtrate is viscous drag as they traverse aqueous channels. The greater the diameter of the molecule relative to that of the channel, the greater the drag.

Water and the common crystalloids of plasma have very small molecular and ionic diameters relative to pore dimensions, of the order of only a few Ångstrom units. Therefore the crystalloids are unrestricted in their entry into pores and in their passage along the pores relative to water. Accordingly, the capillary membrane removes colloids from the filtrate, yet permits free passage of crystalloids and water. At least two ions, calcium and magnesium are in part bound to plasma protein. That fraction which is bound to protein (40 to 60 per cent) is restrained from entering the filtrate; the free fraction dissolved in the aqueous phase traverses the membrane without restriction except for a minor Donnan effect.

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[12]The effective diameter is the Einstein-Stokes diameter, *i.e.*, the diameter of a *sphere which has equivalent diffusion* characteristics. Neither hemoglobin nor albumin is spherical, yet their diffusion properties are the same as spheres of 64 A and 70 A diameter.

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irregular oval apertures from 400 to 900 Å in diameter. The pores, about 1/100 the diameter of a red cell, are of course far too large to account for impermeability of the membrane to colloids. Because of the multiple perforations, the endothelial layer is termed the lamina fenestra.

The middle or basement membrane layer consists of a uniformly dense, apparently structureless, osmophilic tube (the dark-staining layer 3), sandwiched between two concentric tubes of osmophobic material (unstained layers 2 and 4.) Although Hall has described pores in the basement membrane with diameters of the order of 100 Å, others have not observed them, and it is probable that those seen by him were artifacts. The three-layered basement membrane, also called the lamina densa, is some 600 Å thick. It seems likely that this membrane accounts for the colloid impermeability of glomerular capillaries, even though stable pores of the proper dimensions are not demonstrable.

The concept of a pore as a cylindrical channel of fixed dimensions is an operational one. There is evidence that water itself is structured; i.e., the molecules are arranged in a lattice held together by hydrogen bonds. Substances diffuse through water by jumping from position to position in the lattice. It is possible that the basement membrane is a protein-water-phospholipid lattice and that water and solutes traverse it by jumping from position to position, not by bulk flow through continuous cylindrical tubes. If so, it is not surprising that such a tenuous structure cannot be demonstrated by electronmicrography.

The outer layer of the glomerular capillary wall is made up of podocytes (ep. in Fig. 7) and their interdigitating cytoplasmic extensions, the trabeculae and pedicels (layer 1). These podocytes can be considered as "octopus-like" cells sitting on the tube of basement membrane and grasping it with interlocking arms, the trabeculae. The trabeculae carry numerous pedicels which are intimately applied to the lamina densa. They fit closely together leaving the basement membrane exposed through narrow slits. Rhodin believes that these narrow slits between pedicels permit passage of water and crystalloids but restrain colloids. According to this view, protein impermeability would be determined by the

The Electron Microscopic Structure of Glomerular Capillaries unfortunately does not give any clear cut confirmation of the operational picture outlined above. Pores 75 Å in diameter and 600 Å in length should be readily identifiable in electron micrographs were they geometrically stable elements of the capillary wall and were staining methods adequate to distinguish wall substance and pore. It is possible that one or both of these conditions is not met.

According to Hall, Pease, Yamada, and Rhodin, the glomerular capillary is made up of three layers, illustrated diagrammatically in Figure 7. The inner layer consists of the soma and cytoplasmic extensions of endothelial cells. The soma of the endothelial cell,

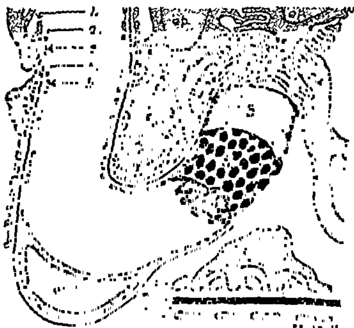


Fig. 7. Schematic representation of the structure of a glomerular capillary as revealed by the electron microscope. (From D. C Pease. *J. Histochem. & Cytochem.* 3:259, 1955.)

containing a large oblate nucleus, lies eccentrically and bulges into the lumen of the capillary. Away from the nucleus, the cytoplasm spreads out into a film some 250 to 500 Å in thickness, forming a continuous endothelial tube (layer 5). This tube is perforated by

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outermost podocyte layer rather than by the basement membrane. Unfortunately no decision can be reached at the moment as to whether the *lamina densa* or the podocyte processes account for selective permeability of the capillary wall. To the author it would appear more reasonable to consider the epithelial cells and their trabeculae as reenforcements, necessitated in glomerular capillaries by high intravascular pressure.

The Rate of Glomerular Filtration averages 125 ± 25 ml. per min. per 1.73 M^2 surface area in the adult male and 110 ± 15 ml. per min, per 1.73 M^2 in the adult female. Filtration rate varies as some function of body size but whether specifically as a function of surface area has never been firmly established. It decreases with advancing age in the absence of cardiovascular, renal or hepatic disease.

Rate of glomerular filtration is a highly significant datum for an understanding of the pathophysiology of renal disease, for the quantification of renal tubular reabsorption and secretion, and for the assessment of the functional defects which underlie the formation of edema. The clearance of inulin is the accepted measure of the rate of glomerular filtration in man and in all other forms studied to date. In the dog, the creatinine clearance is equal to and has the same functional significance as the inulin clearance and is technically a simpler measurement to perform. Unfortunately in man, the creatinine clearance is not identical under all circumstances with the inulin clearance, for a small amount of creatinine is secreted by the renal tubules. Furthermore a part of the apparent endogenous creatinine of plasma is not creatinine and is reabsorbed by the renal tubules.

The concept of renal plasma clearance was developed by Van Slyke and his associates in the course of their investigation of the mechanism of excretion of urea. They defined the urea clearance as the volume of plasma completely cleared of urea by the kidneys in one minute's time. Rehberg first pointed out that the renal plasma clearance of a substance would equal the rate of glomerular filtration if that substance exhibited certain well defined properties. Although he correctly described the requisite properties, the substance he chose, creatinine, failed to exhibit them in man. Some

years later Shannon and Smith and Walker and Richards simultaneously proposed inulin as fulfilling the necessary requirements, a prediction which has been amply confirmed in subsequent investigations.

The Properties of a Substance Whose Clearance Is to Measure Rate of Glomerular Filtration are the following: (1) It must be freely filterable through the pores of the glomerular capillary membrane; i.e., it must be a crystalloid, not bound to plasma proteins and of such dimensions that it is not sieved appreciably in passing through the capillary wall. Inulin with an Einstein-Stokes diameter of 30 Å is about the largest molecule which could freely pass pores 75 Å in diameter without significant sieving. In fact inulin may be sieved to a slight but quantitatively insignificant degree. (2) The substance must be sufficiently large and insoluble in the lipid phase of the tubular epithelium so that it will not be passively reabsorbed. Due to reabsorption of water, inulin becomes highly concentrated in the terminal portions of the renal tubules. Despite high concentration gradients between tubular lumen and peritubular fluid, there is good evidence that inulin does not diffuse back into the bloodstream. (3) The substance must be inert; the renal tubules must neither actively reabsorb nor secrete it. A variety of lines of evidence indicates that inulin meets these requirements. (4) It must be non-toxic, i.e., it must not in itself alter renal function. Providing it is pyrogen-free, inulin meets this requisite. (5) Finally it must be determinable in plasma and urine with a high degree of accuracy. Analytical methods for inulin are satisfactory when carefully applied.

The Principles Involved in the Measurement of Glomerular Filtration Rate are illustrated diagrammatically in Figure 8. Since the kidney consists of many nephrons in parallel, certain functions can be illustrated most simply in terms of a single nephron, many times the size and functional capacity of the actual unit. If 125 ml. of plasma are filtered by this composite glomerulus each minute and if each ml. of plasma contains 1 mg. of inulin, it is obvious that 125 mg. of inulin will be delivered into the renal tubule each minute. Suppose that the urine flow is 1 ml. per min., and that no inulin is reabsorbed and none secreted as the filtrate flows along

the renal tubule. Each minute 125 mg. of inulin will be excreted in 1 ml. of urine. The clearance is defined as the rate of excretion (urine concentration × urine flow) divided by the plasma concentration:

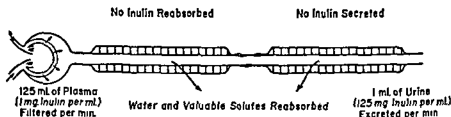


Fig. 8. Principles involved in the measurement of rate of glomerular filtration by the inulin clearance method.

$$C_{in} = \frac{U_{in} \times V}{P_{in}}; \text{ where } C_{in} =$$

clearance of inulin in ml. per min; U_{in} = *urine inulin concentration* in mg. per ml.; V = urine flow in ml. per min.; and P_{in} = *plasma concentration of inulin in mg. per ml. Substituting* the values given above:

$$C_{in} = \frac{125 \text{ mg./ml.} \times 1 \text{ ml./min.}}{1 \text{ mg./ml.}} = 125 \text{ ml. per min.}$$

It is evident that the inulin clearance so calculated is the same as the rate of glomerular filtration assumed in the example cited. *Several factors must be rigorously controlled if measured inulin* clearances are to approximate rates of glomerular filtration satis*factorily:* plasma inulin concentration must be held constant over the period of measurement; urine collections must be complete and accurately timed; no sudden changes in urine flow should occur during the course of clearance measurement; blood samples should be frequent and accurately timed; the analysis of inulin in plasma and urine must be accurate.

It is necessary to point out that equating plasma inulin clearance with glomerular filtration rate is to some extent ambiguous. The inulin clearance is a plasma clearance, yet the aqueous phase, not whole plasma, is filtered through the glomeruli.. If one wishes to determine the volume of plasma water filtered per min., the meas-

ured inulin clearance (C_{in}) must be multiplied by the fraction of water in plasma (normally 0.94 at a plasma protein concentration of 6 per cent).

The Quantities of Ions Filtered per Minute can be readily calculated if one measures simultaneously glomerular filtration rate (C_{in}) and the plasma concentrations of the several ion species (P_{Na}, P_K, P_{Cl} and P_{HCO_3}). The products of these two variables times the appropriate Donnan factor divided by 1,000 (since plasma concentrations of ions are expressed in mEq. per liter) yield the quantities filtered in mEq. per min.[11] The quantities filtered per min. multiplied by 1440 yield the quantities filtered per 24 hrs. Table IV summarizes average values for the quantities of potassium and of the major extracellular ions filtered per minute and per day. In each instance the quantities filtered per day far exceed the quantities present in the extracellular compartment. With the exception of potassium, the quantities filtered also considerably exceed total body stores.

TUBULAR REABSORPTION OF IONS AND WATER

Magnitude of the Reabsorptive Problem. The filtration of ions and water in the copious amounts shown in Table IV demands nearly equivalent tubular reabsorption to prevent rapid exhaustion of body stores. The magnitude of the reabsorptive problem is summarized in the columns on the right of this table. If an individual is in water and electrolyte balance, he must excrete each day in the urine the quantities of ions and water ingested, minus whatever quantities are eliminated by extrarenal routes. The remainder of that filtered must be reabsorbed by the renal tubules. If an individual ingests 2500 ml. of water in food and drink and loses 1000 ml. by extrarenal routes, it is obvious that he must

[11] In calculating quantities of ions filtered, one does not correct for the fraction of plasma which is water. The reason is that ion concentrations are expressed per unit volume of plasma and glomerular filtration rate is expressed as ml of plasma filtered per min. The product of C_{in} (ml. plasma per min) and $P_{Na}/1000$ etc. (mEq. per ml. of plasma) times the Donnan factor gives the quantity of ions filtered directly. However, if one wishes to calculate the concentrations of ions in the filtrate, it is necessary to correct for the difference in water content of plasma and filtrate and for Donnan distribution as well.

TABLE IV

QUANTITIES OF IONS AND WATER FILTERED, EXCRETED, AND REABSORBED BY THE KIDNEYS OF MAN (MEAN NORMAL VALUES)

	Plasma Conc.	*Donnan Factor*	*Glom Filt. Rate*	*Quantity Filtered*		*Quantity Excreted*	*Quantity Reabsorbed*	*Per Cent Filtered Reabsorbed*
	(mEq./L.)		*(ml/min.)*	*(mEq./min.)*	*(mEq./24 hr.)*			
Sodium	140	0.95	125	16.6	23,900	171	23,729	99.3
Chloride	103	1.05	125	13.5	19,500	171	19,329	99.1
Bicarbonate	27	1.05	125	3.55	5,100	2	5,098	99.9
Potassium	4	0.95	125	0.475	684	51	633	80.6
	Plasma Fraction			*(ml/min.)*	*(ml/24 hr.)*			
Water	0.94		125	118	169,000	1,500	167,500	99.1

reabsorb 167,500 ml. of the 169,000 ml. filtered, excreting only 1500 ml. Over 99 per cent of the filtered water is reabsorbed. A luxus intake of sodium chloride is 10 gm. or 171 mMols per day; that of potassium is 2 gm. or 51 mEq. In the absence of sweating or diarrhea, extrarenal losses are minor and may be neglected. The reabsorption of filtered sodium and chloride ions is more than 99 per cent complete, that of potassium, 80 per cent complete. The net dietary intake of bicarbonate is less than zero for the usual diet is acid ash, i.e., it contains more potential acid anions than cations. One and one half liters of urine of pH 5.5 to 6.0 contains about 2 mEq. of bicarbonate. Thus over 99.9 per cent of the filtered bicarbonate is reabsorbed; essentially none is excreted.

ION AND WATER TRANSPORT MECHANISMS IN RENAL TUBULAR CELLS

General Features of Ion and Water Reabsorption. The mechanisms of reabsorption of ions by the renal tubules are no doubt related to, and modifications of mechanisms present in the membrane of every cell. In Chapter II, the characteristic differences in composition of extracellular and intracellular fluids were ascribed to the operation of membrane pumps which extrude sodium from and concentrate potassium within the cell. In most cells, sodium enters by diffusion and is actively extruded through all free surfaces. In renal tubular cells, sodium enters mainly from the tubular lumen and is extruded into the peritubular fluid. The tubular transport system is oriented to pump sodium unidirectionally.

If sodium is pumped from lumen to peritubular fluid, a potential difference will be established across the tubular epithelium which will favor diffusion of anions in the same direction, i.e. downhill along an electrical gradient. Therefore, it is unnecessary to postulate independent active transport mechanisms to account for the reabsorption of the major anions, chloride and bicarbonate. While this thesis will be developed below, it must be emphasized that it has not as yet been established by any rigorous proof.

The active pumping of sodium from lumen to peritubular fluid coupled with the passive diffusion of anions will establish an osmotic force across the tubular epithelium which will favor the

diffusion of water in the same direction. If the tubule is relatively permeable to water, ions and water will be transported at equivalent rates, and although volume of tubular urine will suffer progressive reduction, osmolality will remain unchanged. If, on the other hand, the tubule is impermeable to water, the reabsorption of ions will reduce the osmolality of the tubular fluid causing it to become hypotonic, whereas the immediate peritubular fluid into which ions are pumped will become hypertonic. There is now reason to believe that sodium is actively transported from lumen to peritubular fluid in the proximal segment, in the thin segment of the loop of Henle, in the distal segment and in the collecting duct as well. The proximal tubule is permeable to water under all conditions; therefore, the residual tubular urine remains isosmotic and reabsorption of sodium results in a reduction in volume. The thin segment of the loop of Henle, at least its ascending segment, is impermeable to water; therefore, the residual tubular urine becomes hypotonic, whereas the immediate peritubular fluid becomes hypertonic. The distal tubule and the collecting duct are variably permeable to water. Under conditions of water diuresis, i.e., in the absence of circulating antidiuretic hormone, the distal tubule and collecting duct are impermeable to water; a large volume of hypotonic urine is excreted. Under conditions of hydropenia, i.e., in the presence of a high titre of circulating antidiuretic hormone, the distal tubule and collecting duct are permeable to water; a small volume of hypertonic urine is excreted.

For some time the view has been held that hypertonic urine is formed by the active reabsorption of solute-free water from a small volume of isotonic fluid delivered from the distal segment into the collecting duct. Two theoretical difficulties argue against an active water pump. one, the conceptual difficulty of visualizing a carrier mechanism which will transport water; the other, the extremely rapid turnover required of any carrier complex concerned with water transport. The transport of 1.0 ml. of isotonic saline per min. by a mechanism which actively pumps sodium and which permits osmotic equilibration of water would require roughly 10^{20} successive combinations and dissociations of sodium with carrier. Transport by a mechanism which actively pumps

water and which permits the passive diffusion of sodium would require more than 300 times this number of successive combinations and dissociations of water and carrier. This results from the fact that isotonic saline is 55.5 Molar with respect to water, but only 0.150 Molar with respect to sodium.

Recently Wirz, Hargitay and Kuhn have explained the formation of hypertonic urine in terms of the active transport of sodium from the ascending limb of the loop of Henle into the peritubular interstitial fluid of medulla and papilla, rendering it hypertonic. Water equilibrates across the epithelium of the collecting ducts which traverse this hypertonic tissue; the final urine becomes equally hypertonic. This thesis, revolutionary to say the least, was not generally accepted at first. However, confirmation of its basic tenets by Ullrich, Berliner, Gottschalk and others makes its consideration imperative. We shall develop it in more detail below.

The Basic Characteristics of Ion and Water Transport in the Proximal Tubule have been defined in micropuncture studies begun by Dr. A. N. Richards and his colleagues nearly 30 years ago and continued in recent years by Drs. Bott, Wirz, Gottschalk and others. Clearance studies under conditions of osmotic loading, of water loading, and of altered acid base metabolism have provided much ancillary information.

First, reabsorption in the proximal segment is isosmotic; i.e., solutes and water are reabsorbed at equivalent rates. This statement is based on the original observations of Walker, Hudson, Findley and Richards on the amphibian nephron and those of Walker, Bott, Oliver and MacDowell on the mammalian nephron. It has subsequently been confirmed by Wirz and by Gottschalk. All groups have observed that the glomerular filtrate is isosmotic with plasma and remains so as if flows along the proximal tubule, even though volume is reduced to a fraction of its original value.

Second, ion reabsorption is primary and active; water reabsorption is secondary and passive; i.e. water transport depends largely on osmotic forces set up by the reabsorption of ions. Four lines of evidence support this statement. (1) Wesson and Anslow in experiments on dogs have shown that the rapid infusion of a hypertonic solution of mannitol may increase urine flow to 30 to 40 ml. per

min., i.e., to a value equal to half of filtration rate. At a time when 50 per cent of the water entering the renal tubules in the glomerular filtrate is excreted in the urine, only 8 per cent of the filtered bicarbonate, 22 per cent of the filtered sodium, and 33 per cent of the filtered chloride are excreted. The urine is isotonic[14] and, as claimed by Wesson and Anslow, may represent proximal tubular fluid relatively unmodified[15] during its rapid transit through the distal segment. *If this assumption is correct, then the net reabsorption* of ions in the proximal segment exceeds the net reabsorption of water and at least one and perhaps all of these ions are actively reabsorbed. (2) Solomon and Giebisch have measured potential differences of some 20 to 40 millivolts between tubular lumen (negative) and peritubular fluid (positive) in the Necturus and rat. Such potentials are *most readily explained in terms of active transport of sodium ions, a thesis which will be developed in detail* below. (3) Windhager *et al.* have shown by "stopped flow" perfusion of proximal tubules of Necturus with isotonic mixtures of mannitol and saline in varying proportions that sodium and chloride ions can be actively reabsorbed to establish a gradient of 2:3 between *lumen and peritubular fluid.* (4) *In preliminary micropuncture* studies on the rat under conditions of mannitol diuresis, Giebisch and Windhager have found that sodium and chloride ions are reabsorbed against significant concentration gradients, as great as 1·2. Points (3) and (4) above constitute proof of active transport of ions across the proximal tubule. Since the tubular fluid remains isotonic, i.e. since the concentration of mannitol is increased in proportion to the reduction in concentration of ions, it is reasonable to conclude that water is reabsorbed osmotically and secondary to the reabsorption of ions.

Third, reabsorption is essentially isohydric under normal conditions; the pH of the glomerular filtrate changes little as it flows along the proximal segment. This fact has been directly demonstrated by Montgomery and Pierce only for the amphibian neph-

[14]The osmotic deficit created by the reabsorption of ions was exactly balanced by the increase in mannitol concentration which resulted from water reabsorption.
[15]The low bicarbonate content of the urine may in part be due to rapid reabsorption in the distal tubule. Hence "unmodified proximal tubular fluid" can be applied to the final urine only with significant reservations.

ron. In the absence of evidence to the contrary, it has been inferred for the mammalian nephron. Results of Ullrich et al and of Pitts et al are consonant with this view, for they indicate that the site of major acidification in the mammalian as well as in the amphibian nephron is distal. If proximal reabsorption is isohydric, bicarbonate and water must be reabsorbed at equivalent rates; i.e. no significant change in bicarbonate concentration occurs in the proximal segment during reabsorption of the bulk of the filtrate. No doubt the proximal epithelium permits the free diffusion of carbon dioxide. If bicarbonate concentration and pCO2 remain unchanged, pH is also unchanged.

It has recently been shown by Gottschalk that in marked osmotic diuresis in the rat, produced by the infusion of 25 per cent glucose, proximal tubular fluid may become 0.6 to 0.8 pH units more acid than blood plasma, an observation confirmed by Giebisch and Windhager in mannitol diuresis as well. However, these latter investigators have shown that in diuresis induced by the infusion of isotonic saline, no significant acidification of proximal fluid occurs. As has been noted above, a sodium gradient as great as 1:2 is established between tubular fluid and peritubular blood plasma in profound osmotic diuresis. One would predict an equivalent anion gradient, and Giebisch and Windhager have observed one for chloride roughly equal to that for sodium. If a comparable bicarbonate gradient were also established, the pH of proximal urine would be 0.3 to 0.4 units below that of plasma. In contrast, in saline diuresis no significant sodium or chloride gradient develops across the proximal tubule. One would, therefore, predict no bicarbonate nor pH gradient and none of appreciable magnitude has been found.

The condition of saline diuresis more closely approximates the normal than does osmotic diuresis, for little or no sodium gradient is established under normal conditions or in saline diuresis, whereas an appreciable gradient develops in osmotic diuresis. However, it should be pointed out that the degree of proximal acidification in osmotic diuresis is roughly twice that explainable on the basis of a bicarbonate gradient equal to that for sodium. Accordingly, bicarbonate must be preferentially reabsorbed with respect to

either sodium or chloride in osmotic diuresis. Furthermore, Giebisch and Windhager have shown that in saline diuresis combined with respiratory acidosis, proximal tubular urine is acidified to a degree equivalent to that observed in osmotic diuresis. Therefore under two conditions, osmotic diuresis and respiratory acidosis, bicarbonate is preferentially reabsorbed and the tubular urine acidified. In saline diuresis under conditions of normal acid base balance and probably in non-diuretic normal conditions as well, no preferential reabsorption of bicarbonate and no acidification of the urine occurs. The significance of these findings with respect to the mechanism of bicarbonate reabsorption is not clear at the moment.

Fourth, under normal conditions, i.e., in the absence of osmotic diuresis, no significant concentration gradients are established between proximal tubular fluid and plasma for any of the three ions, sodium, chloride and bicarbonate. This view is based on micropuncture studies on both amphibian and mammalian nephrons. Most would agree that the sodium concentrations of proximal tubular fluid and of plasma are essentially equal. Recent work of Giebisch and Windhager has demonstrated that the chloride concentrations are the same. The observations, described in the paragraphs above, suggest that the bicarbonate concentrations are also the same. However, it must be reiterated that identity of concentration of ions in proximal fluid and plasma holds only under normal conditions and in saline diuresis, not in osmotic diuresis, and not in respiratory acidosis.

Fifth, there occurs a marked reduction in volume of the tubular contents as fluid flows along the proximal segment of the mammalian nephron. According to Walker, Bott, Oliver and MacDowell, some 4/5ths and perhaps, as Smith claims, as much as 7/8ths of the ions and water are reabsorbed.

The characteristics of proximal tubular reabsorption of ions and water outlined above are implicit in the diagram of Figure 9. Thus reabsorption of sodium is active, that of water is passive and dependent on osmotic forces set up by the reabsorption of sodium. The glomerular filtrate is isotonic with plasma and the tubular fluid remains isotonic as it flows along the convoluted and straight

portions of the proximal segment. Volume at the end of the proximal tubule is reduced to a quantitatively uncertain degree, but probably to a value within the range of 1/5th to 1/8th that of the glomerular filtrate. If we assume that 125 ml. of plasma are filtered through the glomeruli each minute, 118 ml. of plasma water per minute would be delivered into the proximal segments of the renal tubules. Sodium ions along with chloride and bicarbonate ions in equivalent quantities are reabsorbed. Glucose, phosphate, uric acid, amino acids, vitamins and other normal constituents of the blood plasma are more or less completely reabsorbed. Water is osmotically reabsorbed and volume is reduced to perhaps 15 to 20 ml. per min. at the end of the proximal tubule.

It is well to reemphasize here that the osmolality of the plasma and glomerular filtrate is in large part due to its content of sodium, chloride and bicarbonate ions. Therefore, it is the reabsorption of these ions which creates the major fraction of the osmotic force that causes water reabsorption. A minor fraction is contributed by the reabsorption of glucose, amino acids, phosphate and sulfate. Excretory products such as urea, creatinine, uric acid, etc. are concentrated to some degree by the reabsorption of fluid in the proximal segment. Theoretically they would be concentrated some 5 to 8 times, practically somewhat less, for a fraction of the filtered urea (according to Shannon, some 20 to 40 per cent) diffuses back into the blood across the proximal tubular epithelium. Obviously, these excretory products exert a greater proportion of the osmotic pressure of the tubular fluid at the end of the proximal segment than they do at its beginning. Accordingly sodium, chloride and bicarbonate ions must exert a somewhat smaller proportion of total osmotic pressure; i.e., their concentrations must be slightly lower at the end than at the beginning of the proximal tubule.

An Alternative View of Passive Reabsorption of Proximal Fluid. Bayliss and more recently Malvin et al have suggested that proximal reabsorption of fluid (water and sodium and chloride ions) is passive and due to the colloid osmotic force exerted by the plasma proteins in the peritubular capillaries and the crystalloid osmotic force developed in consequence of the active reabsorption of glucose, amino acids, and other so-called threshold solutes. This

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when the urinary load of excretory solutes is within the usual normal range. However, in profound osmotic diuresis induced by the infusion of mannitol, the concentration of osmotically active excretory products in the glomerular filtrate may be increased from a normal value of 6 or 7 mOsm. per liter to 60 or more mOsm. per liter. If the only force available to reabsorb fluid in the proximal tubule were that represented by the 7.0 mOsm. per liter of peritubular colloids and actively reabsorbed threshold solutes, 10 per cent or less of the filtrate could be returned to the blood stream. The reabsorption of 10 per cent of the fluid would concentrate the mannitol by 10 per cent and the process would stop. No gradient of concentration of sodium and chloride ions could be established by such a mechanism.

As mentioned earlier, Windhager *et al.* and Giebisch *et al.* have observed that a gradient of concentration of sodium and chloride ions develops along the proximal tubule of both the Necturus and rat when mannitol is present in the tubular fluid. Such a gradient permits the reabsorption of considerably more fluid than the 10 per cent noted above. Furthermore, the development of a gradient demands active transport of one or both ions.

The basic question is not whether the above mentioned oncotic and osmotic forces, equivalent to 7.0 mOsm. per liter, contribute to the reabsorption of fluid, obviously they must, but whether the tubular epithelium is so freely permeable to ions that they are unrestricted in their movements relative to water, hence exert no osmotic effects. The author believes that evidence available to date favors the view that active transport of sodium is necessary to effect the reabsorption of the bulk of the filtrate in the short time that the fluid is in contact with proximal tubular epithelium. The extent to which passive diffusion of ions and water occurs down the slight oncotic and osmotic gradient contributed by plasma proteins and by reabsorbed threshold substances is undetermined. The bidirectional fluxes of sodium, potassium, and chloride across the tubule of the frog and Necturus observed by Hoshiko et al, Chinard *et al.*, Whittenbury *et al.*, and Giebisch and Windhager suggest that the tubule may be sufficiently permeable to these ions to permit some passive reabsorption of proximal fluid. That such

implies that the permeability of the proximal epithelium to sodium and chloride ions is of the same order of magnitude as the permeability to water, and is roughly comparable to that of the glomerular capillary membrane. This is equivalent to saying that only the excretory products in the tubular fluid exert an osmotic effect;

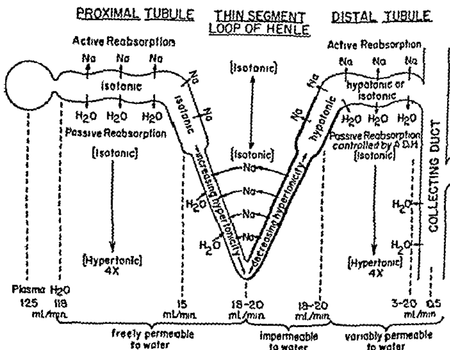


Fig 9. The functional organization of the nephron in relation to reabsorption of sodium and water and formation of dilute and concentrated urine The diagram incorporates views of Wirz, Hargitay, and Kuhn, Gottschalk, and Berliner

the tubule is freely permeable to or actively reabsorbs all other solutes. Were this true, fluid could be reabsorbed passively until the excretory products are concentrated some 7 mOsm. per liter above their concentration in the glomerular filtrate. This derives from the fact that the colloid osmotic force of the plasma proteins in the peritubular capillaries is equivalent to 2.0 mOsm. per liter and the crystalloid osmotic force of actively reabsorbed threshold solutes is equivalent to 5.0 mOsm. per liter. Were all assumptions valid, such a passive mechanism could theoretically account for the reabsorption of half to two-thirds of the glomerular filtrate

when the urinary load of excretory solutes is within the usual normal range. However, in profound osmotic diuresis induced by the infusion of mannitol, the concentration of osmotically active excretory products in the glomerular filtrate may be increased from a normal value of 6 or 7 mOsm. per liter to 60 or more mOsm. per liter. If the only force available to reabsorb fluid in the proximal tubule were that represented by the 7.0 mOsm. per liter of peritubular colloids and actively reabsorbed threshold solutes, 10 per cent or less of the filtrate could be returned to the blood stream. The reabsorption of 10 per cent of the fluid would concentrate the mannitol by 10 per cent and the process would stop. No gradient of concentration of sodium and chloride ions could be established by such a mechanism.

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Ion Transport in the Thin Segment of the loop of Henle. A series of rather surprising observations, at first difficult to accept, but now amply confirmed, has necessitated a revision of concepts of the function of the thin segment of the loop of Henle. A bit of historical review may help to give perspective.

Marshall and his colleagues a number of years ago pointed out the following two facts: only birds and mammals have a true loop of Henle interposed between proximal and distal convoluted tubules; only birds and mammals can form urine hypertonic to blood plasma. They postulated that the urine is concentrated in the thin segment of the loop of Henle by the active reabsorption of water, and that water transport is specifically stimulated by antidiuretic hormone. If this view were correct, urine collected by micropuncture from any portion of the nephron distal to the thin segment should be as hypertonic to plasma as is the final urine.

Subsequently, it was observed that the urine collected from the distal convoluted tubules may be isotonic or even hypotonic when the ureteral urine is hypertonic; concentration must therefore be the final step in the elaboration of urine and must take place in the collecting ducts. Smith postulated that the thin segment of the loop of Henle plays only a passive role, ensuring osmotic equilibration of proximal tubular urine with blood plasma prior to its delivery into the distal segment. According to Smith the urine is concentrated in the collecting ducts by the active reabsorption of water from a small volume of isotonic fluid.

Certain rodents, especially the desert rat, can form urine which is far more hypertonic to the plasma than is that of dog and man. These forms have a long urinary papilla, made up of thin segments of loops of Henle interspersed with collecting ducts and capillary loops, which projects into a greatly elongated renal pelvis. Evidence has recently accumulated that the medullary tissue and especially the urinary papilla are very significantly hypertonic to the renal cortex and to other tissues. Thus Glimstedt and Ljungberg noted that slices of medullary tissue taken in succession from the corticomedullary junction to the tip of the urinary papilla

contain increasing amounts of chloride. Wirz, Hargitay and Kuhn observed that the osmotic pressure of such slices progressively increases to reach a maximum at the tip of the papilla. Ullrich noted that urea is highly concentrated in the papilla and Levinsky made similar observations with respect to sodium. Finally Wirz noted that blood collected from surface capillaries of the papilla is hypertonic to general systemic blood. The hypertonicity of medullary and papillary tissue cannot be ascribed solely to hypertonic urine contained in the collecting ducts. The tissue itself must be hypertonic. The thesis of Wirz and more recently that of Gottschalk is that sodium pumps located in the thin segments of the loops of Henle extrude sodium ions (plus equivalent numbers of chloride ions) into the interstitium of the medulla and urinary papilla rendering it hypertonic to plasma. Water is abstracted osmotically from the urine in the collecting ducts until its osmotic pressure increases to equal that of the surrounding papillary interstitial fluid.

Counter-Current Multiplication of Concentration in the Loops of Henle. The details of loop function, shown in the center section of Figure 9, follow in general the concept of Wirz, Hargitay and Kuhn and of Gottschalk. The concept is that of a counter current concentration multiplier. Details of operation are illustrated in Figure 10. Isotonic urine is delivered into the descending limb of the loop of Henle from the proximal tubule. From the corresponding level of the ascending limb, sodium is actively extruded into the interstitium, reducing concentration in the ascending limb, increasing concentration in the interstitium. According to Wirz, sodium may be actively secreted into the descending limb; according to Gottschalk it merely diffuses into the descending limb down a small gradient of concentration. The epithelium of the ascending limb from the tip of the loop well into the cortex must be impermeable to water, otherwise water would follow sodium osmotically and no change in concentration would result. This feature is indicated in Figures 9 and 10, by the heavy line forming the wall of the ascending limb. The descending limb, in contrast, is probably permeable to water.

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The significant event as one follows the two limbs of Henle's loop from the cortico-medullary junction to the tip of the papilla is the transfer of sodium from ascending limb to descending limb, active extrusion from the ascending limb and either active secretion or passive diffusion into the descending limb. The significant

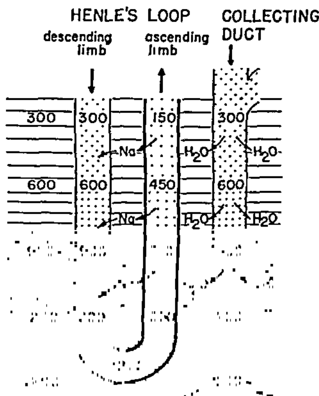


Fig. 10. The role of counter current multiplication of concentration in the loop of Henle in the elaboration of hypertonic urine.

consequences of cyclic recirculation of sodium are: progressively increasing osmolar concentration as the urine flows down the descending limb; progressively decreasing concentration as urine flows up the ascending limb; and progressively increasing osmolality of the interstitial fluid from the cortico-medullary junction to the tip of the papilla. An especially intriguing feature of this hypothesis is that at no point within the system must sodium be

pumped against a high concentration gradient. The maximum osmotic gradient (4 to 1 for the human kidney) is developed longitudinally over the entire length of Henle's loop. The diagrams of Figures 9 and 10 give an erroneous impression of the volume of interstitial fluid in the papilla. In histological section, volume is minimal; tubules and capillaries are densely packed. Therefore, minimal amounts of sodium need be sequestered in the interstitial fluid of medulla and papilla to render it hypertonic.

As was pointed out above, hypertonicity of medullary and papillary tissue has been demonstrated by several investigators. Wirz has shown that blood collected from papillary capillaries is hypertonic. Gottschalk has recently demonstrated by micropuncture of loops of Henle near the papillary tip that the tubular urine is equally hypertonic. The basic elements of the thesis of Wirz and Gottschalk have, therefore, been established directly. At present there is no valid means of determining whether transfer of sodium into the descending limb is active (Wirz) or passive (Gottschalk). The system proposed by Wirz would operate more effectively in that the sodium pumps of the ascending and descending limbs of Henle's loops would operate in series, the system proposed by Gottschalk does not involve active reabsorption in one limb of Henle's loop and active secretion in the other, an assumption which is philosophically somewhat hard to accept.

Counter-current Exchange in Capillary Loops of the Papilla. One might reasonably predict that the flow of blood in capillaries supplying the medulla and papilla would so rapidly dissipate the hyperosmolality of tissue and interstitial fluid that it would be ineffective as a means of concentrating the urine during final transit through the collecting ducts. Wirz first suggested that the arrangement of capillaries in the medulla and papilla is such as to permit their functioning as counter-current exchangers of diffusible solutes. Counter-current exchange considerably reduces the effect of blood flow in dissipating the osmotic gradient. Furthermore, it is probable that only a minor fraction of total renal blood flow perfuses the medulla and papilla. Low total flow and counter-current exchange are both important for the maintenance of hyperosmolality of medullary and papillary tissue.

Berliner has illustrated the operation of the counter-current vascular loop in terms of a thermal model presented in Figure 11, A. B. Consider the tube shown in diagram A on the left, through which water flows at a constant rate of 10 ml. per min. A source supplies heat at a rate of 100 calories per min. If the fluid entering the system has a temperature of 30°C., that leaving the system will have a temperature of 40° C. If the tube is bent upon itself, as shown in diagram B, insulated to prevent heat loss to the outside, but so arranged as to permit free transfer of heat between emergent and entering streams, certain features of operation will

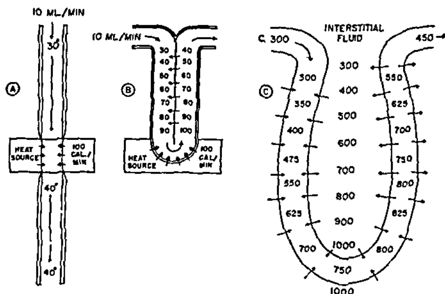


Fig. 11. The principal of counter current exchange. A. Thermal model without counter current exchange. B. Thermal model incorporating counter current exchange. *C. Counter current exchange in medullary capillary loops as a means of preservation of tissue hypertonicity.* (From R.W. Berliner, N.G. Levinsky, D G. Davidson, and M Eden· *Am. J. Med.*, 24 730, 1958.)

change. If rate of fluid flow, the addition of heat, and the temperature of the entering stream are the same, the temperature of the emergent stream will also be the same. However, the temperature at the heat source will be much higher in the counter-current system, for some of the heat will be transferred from the outflowing to the inflowing streams. If one considers the function of the stream of water to be that of cooling the heat source, it is

evident that the straight through system on the left is more effective than the counter-current system. In other words, so far as its cooling effect on the heat source is concerned, the counter-current arrangement has reduced the effective flow to a small fraction of the actual flow.

The capillary shown in diagram C on the right illustrates the operation of the counter-current loop in terms of its effect in preserving the osmotic gradient in the renal medulla. Blood enters the loop with an osmotic concentration of 300 mOsmols per liter. As the capillary dips into the medullary and papillary interstitium with its high osmolal concentration, osmotically active particles diffuse into the blood. As blood traverses the loop and ascends, osmotically active particles diffuse out into the interstitium. The loop operates to reduce the effective blood flow with respect to dissipation of the interstitial osmotic gradient. Furthermore, total blood flow in the papilla is probably low. These two factors are significant in permitting the development of, and for the maintenance of an osmotic gradient.

Ion and Water Transport in the Distal Tubule. Walker, Bott, Oliver and MacDowell first showed by micropuncture of the nephron of the rat that the fluid delivered into the first part of the distal tubule is hypotonic to plasma even when the final urine is hypertonic. This fact was later confirmed by Wirz and by Gottschalk (*cf.* Fig. 9). If we accept the hypothesis of Wirz that sodium is actively transported into the descending limb of Henle's loop, then most of the water reabsorbed from the collecting ducts into the papillary interstitium will follow the sodium osmotically. Volume flow in the ascending limb of the loop and in the first part of the distal segment may be estimated to be 18 to 20 ml. per min. It will be greater than the volume entering the loop of Henle from the proximal tubule by the amount of water reabsorbed from the collecting ducts. On the other hand, if we accept the hypothesis of Gottschalk that sodium diffuses passively into the descending limb of Henle's loop, then the water reabsorbed from the collecting ducts will largely enter the blood capillaries of the medulla and papilla; the volume leaving the loop of Henle may not differ greatly from the volume entering it. The point of

Berliner has illustrated the operation of the counter-current vascular loop in terms of a thermal model presented in Figure 11, A, B. Consider the tube shown in diagram A on the left, through which water flows at a constant rate of 10 ml. per min. A source supplies heat at a rate of 100 calories per min. If the fluid entering the system has a temperature of 30°C., that leaving the system will have a temperature of 40° C. If the tube is bent upon itself, as shown in diagram B, insulated to prevent heat loss to the outside, but so arranged as to permit free transfer of heat between emergent and entering streams, certain features of operation will

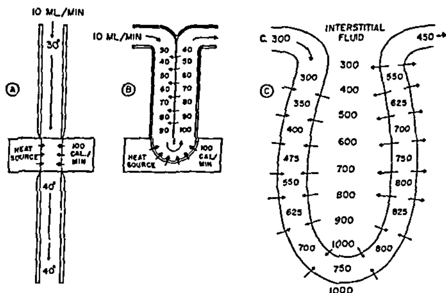


Fig. 11. The principal of counter current exchange. A. Thermal model without counter current exchange. B. Thermal model incorporating counter current exchange. C. Counter current exchange in medullary capillary loops as a means of preservation of tissue hypertonicity. (From R.W. Berliner, N.G Levinsky, D.G. Davidson, and M. Eden. *Am. J. Med.*, 24.730, 1958.)

change. If rate of fluid flow, the addition of heat, and the temperature of the entering stream are the same, the temperature of the emergent stream will also be the same. However, the temperature at the heat source will be much higher in the counter-current system, for some of the heat will be transferred from the outflowing to the inflowing streams. If one considers the function of the stream of water to be that of cooling the heat source, it is

As it flows along the collecting duct the tubular fluid gives up water to the medullary and papillary interstitium. Volume is reduced to 0.3 to 0.5 ml. per min. and osmolar concentration is increased to 1200 to 1400 mOsmols per liter, essentially that of the papillary tissue.

NATURE OF MECHANISMS FOR TUBULAR TRANSPORT OF IONS

In all of the preceding discussion it is implied if not explicitly stated that the tubular transport of sodium is primary and active, that of chloride and bicarbonate secondary and passive. We may loosely define active transport of an ion as net movement of that ion from a region of lower to a region of higher electrochemical potential; passive transport, as net movement from a region of higher to a region of lower electrochemical potential. While this definition by no means covers all possibilities, it is adequate for our purposes. That sodium transport by the renal tubules is active, i.e., uphill against an electrochemical gradient, is highly probable. That chloride and bicarbonate transport is downhill along an electrical gradient will become apparent when we consider potential differences between tubular lumen and bloodstream. That there is no active transport of these anions has not been firmly established. However, the assumption of active sodium reabsorption and passive chloride and bicarbonate reabsorption is reasonable, for it brings the basic transport mechanisms of renal tubular cells into line with those of red cells, muscle cells and nerve cells. Smith, Weston, Berliner and others have suggested this possibility. We shall develop the thesis in somewhat more detail, borrowing liberally from the transport mechanism of frog skin outlined by Ussing and from those of red cells, muscle cells and nerve cells described by Shaw, Glynn, Hodgkin, Keynes and others.

Ussing has pointed out that certain purely physical forces apart from active transport can operate to cause the net transfer of ions across membranes. These include, (1) differences in concentration, (2) differences in activity coefficients, (3) differences in electrical potential between phases in contact with the membrane and

difference is an academic one and it makes little difference which view is accepted. The degree of hypotonicity of the fluid entering the first part of the distal tubule will be conditioned by the rate of uptake of solute free water from the collecting ducts and by the rate of loss of salt from the medullary interstitium to blood flowing in the papillary capillary loops. Counter-current exchange of solute in capillary loops is of course not completely effective and any salt lost to the blood must be replaced from the fluid in the ascending limb of Henle's loop. Since the tubular fluid remains hypotonic to a point where the convolutions of the distal segment approach the parent glomerulus, it is evident that the epithelium to this point must be impermeable to water.

As shown in Figure 9, sodium reabsorption continues in the distal tubule. In the maximally hydrated individual, the concentration of circulating antidiuretic hormone is minimal and the distal tubule and collecting ducts are impermeable to water. Continued reabsorption of sodium reduces concentration to a very low value and a large volume of dilute urine is excreted. The rate of urine flow will be roughly the same as the rate at which proximal tubular fluid is delivered into the descending limb of Henle's loop. *Relatively little fluid will be gained in the loop of* Henle, relatively little lost in the distal tubule and collecting duct.

In the hydropenic individual, the concentration of circulating antidiuretic hormone is high and both distal tubules and collecting ducts are permeable to water. Hypotonic urine delivered into the distal segment loses water to the cortical interstitium. Continued reabsorption of sodium creates an osmotic force which causes further reabsorption of water. Volume is reduced from 18 to 20 ml. per min. at the beginning of the distal tubule to 3 to 6 ml. per min. at the end. The tubular fluid is isotonic as it enters the collecting ducts and contains most of the products destined for excretion.[14]

[14] Ullrich and his associates have recently shown by a remarkably ingenious method [illegible] is reabsorbed [illegible] ammonia and [illegible] of the orifices of collecting ducts at the tip of the papilla with filamentous polyethylene tubes. Minute samples of urine were collected at various levels as the catheters were advanced.

the continued inward diffusion of sodium from the tubular lumen. One view would be to consider that the sodium transport mechanism is an electrogenic ion pump; i.e. the pumping of sodium directly establishes the potential difference, positive outside, negative inside. If this were true, potassium would migrate into the

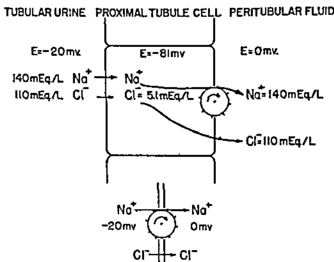


Fig. 12. Hypothetical mechanism of transport of sodium and chloride ions by proximal tubular cells. (From R.F. Pitts *Am. J. Med.*, 24.745, 1958.)

cell along this electrical gradient until the high ratio of intracellular to extracellular potassium concentration would just balance the potential difference. Another view, more in line with current thinking on frog skin, nerve, and muscle and illustrated in Figure 3 A, B would be that the sodium pump is electrically neutral due to coupling of inward movement of potassium with extrusion of sodium. The transcellular potential then becomes a potassium diffusion potential. No net flux of potassium need occur if passive outflux were to equal active influx; i.e., if the cell were to leak potassium back into the peritubular fluid as rapidly as it transports sodium.

Let us further assume that chloride is free to diffuse out of the cell into the peritubular fluid. If diffusion is free, one can calculate from the Nernst equation and the potential difference of 81 milli-

finally, (4) solvent drag force, arising from passage of solvent through the membrane. None seems capable of contributing to an understanding of the overall mechanism of active sodium transport. At least one, namely differences in electrical potential between phases in contact with the membrane, probably plays an important role in the reabsorption of chloride and bicarbonate. Solvent drag may play a role in the reabsorption of all components of the filtrate not reabsorbed solely by carrier mechanisms.

Wilbrandt, Sidney Solomon and more recently Giebisch, utilizing microelectrodes, have recorded potentials between the tubular lumen and peritubular fluid, the so-called transtubular potentials. In addition Giebisch has recorded potentials between the interior of proximal tubular cells and the peritubular fluid, the so-called transcellular potentials. Both transtubular and transcellular potentials play key roles in the thesis we wish to develop. The only complete measurements of both potential values are those of Giebisch on the amphibian kidney; however, we shall make the extrapolation, partially justified by Solomons measurements, that equal or higher potential differences occur in the mammalian kidney. In any event the reader should realize that the hypothesis we shall advance is tentative and represents merely a personal synthesis of evidence too fragmentary and incomplete to constitute proof.

Nature of Proximal Tubular Transport of Sodium and Chloride. According to Giebisch there exists a transcellular potential difference of 60 to 90 millivolts between the interior of proximal tubular cells and peritubular fluid in the kidney of Necturus. The interior of the cell is negative to its surroundings. A transtubular potential difference of around 20 millivolts exists between tubular lumen and peritubular fluid. The lumen of the tubule is negative to the peritubular fluid. The relationships of these potentials are represented diagrammatically in Figure 12.

Let us assume that the transcellular potential is basically established by a pump which actively extrudes sodium from the cell into the peritubular fluid. The pump operates continuously and at such a rate that the sodium content of the cell is maintained at a low value, perhaps 1/10th or less that of extracellular fluid, despite

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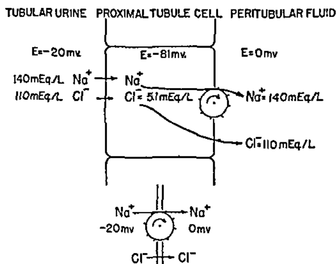


Fig. 12. Hypothetical mechanism of transport of sodium and chloride ions by proximal tubular cells. (From R.F. Pitts: *Am. J Med.*, 24 745, 1958)

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diffuse from cell to tubular lumen are buffered by bicarbonate ions to form carbonic acid. The carbonic acid dehydrates to CO_2 and water, the CO_2 diffusing into the cell to re-enter the bicarbonate cycle. Since sodium and water are reabsorbed and bicarbo-

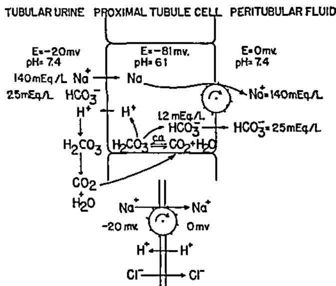


Fig 13. Hypothetical mechanism of transport of sodium and bicarbonate ions by proximal tubular cells. (From R.F. Pitts *Am. J. Med.*, 24 745, 1958.)

nate disappears at equivalent rates, the bicarbonate concentration and pH of the residual fluid will remain unchanged.

The simplified diagram at the bottom of Figure 13 illustrates the overall operation of the proximal ion reabsorptive system. Sodium is actively transported from lumen to peritubular fluid. Chloride diffuses passively in the same direction, but since its diffusion is restricted relative to sodium transport, the transtubular potential difference of 20 millivolts develops. Hydrogen ions diffuse into the tubular lumen along this electrical gradient.

Many readers may legitimately enquire why such a complicated mechanism need be postulated to explain reabsorption of bicarbonate if the rather simple one of movement down an electrical gradient suffices for chloride. The answer is that a number of

volts that the intracellular chloride concentration will be low, roughly 5 mEq. per liter. If one further assumes that there is some restriction to the diffusion of chloride into the cell from the tubular lumen relative to sodium, one can account for the transtubular potential of minus 20 millivolts. This is clearer in the simplified diagram at the bottom of Figure 12, in which the tubular epithelium is represented as a single membrane. Active transport of sodium accompanied by passive diffusion of chloride will lead to the development of a transtubular potential of proper sign, if chloride diffusion lags behind sodium transport. Were chloride to diffuse as fast, it would short circuit the sodium pump and no transtubular potential would exist. We feel that this transtubular potential plays an important role in the sodium-hydrogen exchange process which Berliner, Schwartz, Gilman, and Pitts believe to be important in the reabsorption of bicarbonate in the proximal tubule. One obvious ambiguity in Figure 12 is that we have cited amphibian potentials and mammalian ion concentrations. This is done purposely to emphasize the fact that we are illustrating a concept, not attempting to prove a thesis.

Nature of Proximal Tubular Transport of Bicarbonate. The cell represented in Figure 13 might well be the same one shown in Figure 12. However in this second diagram, sodium and bicarbonate reabsorption rather than sodium and chloride reabsorption is illustrated. The sodium pump establishes the transcellular potential of 81 millivolts. Let us assume that the luminal border of the cell is essentially impermeable to bicarbonate ion but that the peritubular border is as permeable to bicarbonate as to chloride. We can then calculate that the internal bicarbonate concentration will be low, of the order of magniture of 1.0 mEq. per liter. This is equivalent to the Boyle and Conway formulation of the Donnan distribution of bicarbonate in muscle. If the cell is permeable to dissolved CO_2, the pH of its contents will be roughly 6.1. Hydrogen ions are therefore more than 20 times as concenrated within the cell as in the tubular urine. They will diffuse outward from the cell into the tubular lumen despite the net electrical gradient of 61 millivolts opposing diffusion. No active transport of hydrogen ions would necessarily be required. The hydrogen ions which

ever, the observation of modest acidification of proximal tubular urine does not demand active transport of hydrogen ions.

Nature of Distal Tubular Transport of Sodium, Hydrogen and Potassium. The distal transport mechanisms are undoubtedly much more complicated than the proximal mechanisms just described. This statement derives from the following facts. First, in forming urine of pH 4.4 from plasma of pH 7.4, a process now definitely localized to distal portions of the nephron in both the amphibian and mammalian kidney, hydrogen ions must be transported against a gradient of 1,000 to 1. Were this a passive transfer, it would demand a transtubular potential of the order of 180 millivolts, a value far in excess of any measured by Solomon or Giebisch in either the rat or Necturus. Second, in forming dilute urine, distal tubular cells can remove sodium almost completely from the tubular fluid, certainly against a gradient of 50 to 1. Third, as Berliner, and others have shown, hydrogen ions and potassium ions compete for the sodium exchange mechanism.

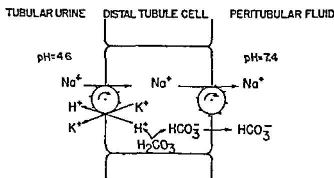
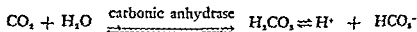


Fig. 14. Hypothetical mechanism of distal tubular and/or collecting duct cells concerned with reabsorption of sodium ions in exchange for hydrogen ions and potassium ions. (From R.F. Pitts. *Am. J. Med.*, 24 745, 1958)

These three facts prompt us to postulate the existence of two sodium pumps in the distal tubular cell as shown in Figure 14. One, located in the luminal membrane, is a coupled ion exchange pump, transporting sodium ions into the cell in exchange for either hydrogen or potassium ions. However, this pump transports more sodium in an inward direction than it does hydrogen and potas-

lines of evidence suggest that bicarbonate is reabsorbed indirectly, and that the mechanism involves hydrogen for sodium exchange coupled with carbon dioxide diffusion. Inhibitors of the enzyme carbonic anhydrase partially block bicarbonate reabsorption, implying that the reaction



$$CO_2 + H_2O \overset{\text{carbonic anhydrase}}{\rightleftharpoons} H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

is a significant step in the transport process. An increase in pCO_2 of the blood, which drives the reaction to the right and increases the hydrogen ion concentration within the tubular cells, facilitates the reabsorption of bicarbonate. Lowering the pCO_2 of the blood by hyperventilation inhibits reabsorption. Were bicarbonate reabsorbed as a stable ion species as is chloride, one would not expect transport to be affected by the above mentioned factors.

Some readers may object to the thesis outlined in Figure 13 on the basis of the low pH of the interior of the tubular cell. Although Boyle and Conway and Conway and Fearon maintain that the intracellular pH of muscle is 6.0, Wallace and Hastings and Gardner, MacLachlan and Berman claim it is between 6.4 and 6.9. Anderson and Mudge calculate that the pH of the interior of cells of renal cortical slices varies from 7.0 to 7.4. Actually it is not necessary for the pH of proximal cells to be as low as 6.1 for hydrogen ions to diffuse from cell contents to lumen.

If the transtubular potential is greater than 20 millivolts, the pH of the tubular cell could be higher than 6.1 and passive diffusion of hydrogen ions might still occur. The value of 20 millivolts for transtubular potential applies to the amphibian kidney; potentials as high as 39 millivolts have been recorded from proximal tubules of rats. It is possible, therefore, for the cell pH to be within a more reasonable range, yet passive diffusion of hydrogen ions might still occur. That transport is passive is far from proven.

Indeed recent observations of Gottschalk, Giebisch, and Windhager that the urine may be acidified in the proximal segment in osmotic diuresis suggest the possibility of active transport of hydrogen ions. Differences between proximal and distal ion transport mechanisms may be more quantitative than qualitative. How-

Hoeber had shown that acidification is depressed by sulfanilamide, an inhibitor of the enzyme carbonic anhydrase. Pitts and Alexander found that sulfanilamide markedly reduced the excretion of titratable acid by the acid and phosphate loaded dog. They interpreted these results in terms of the diagram presented in Figure 15A.

Alkaline dibasic sodium phosphate enters the renal tubule in the glomerular filtrate and is converted in the distal segment into acid monobasic sodium phosphate by the exchange of one hydrogen ion for one sodium ion per molecule of phosphate. The hydrogen ions are derived from carbonic acid, formed within distal tubular cells by the hydration of carbon dioxide, a reaction which is catalyzed by carbonic anhydrase. In the presence of enzyme inhibitors such as sulfanilamide, hydration of carbon dioxide to carbonic acid slows, and the rate at which hydrogen ions are presented to the exchange mechanism is reduced. The excretion of titratable acid is depressed. Under normal conditions, the hydrogen ions exchanged for sodium ions are excreted as monosodium-dihydrogen phosphate, i.e., as titratable acid. The sodium ions are restored to the blood stream along with equivalent numbers of bicarbonate ions. Pitts and Alexander pointed out that the exchange of hydrogen ions for sodium ions in the formation of acid urine is an energy consuming process. Figure 14 illustrates this fact in terms of a coupled ion pump located in the luminal membrane of the tubular cell and concerned with the active extrusion of hydrogen and the active reabsorption of sodium ions

Exchange of Hydrogen Ions for Sodium Ions in the Distal Tubular Reabsorption of Sodium Bicarbonate was proposed a year later by Pitts and Lotspeich. This concept is illustrated in Figure 15B. Under normal conditions, much more bicarbonate than phosphate enters the distal tubules. The exchange of hydrogen for sodium converts bicarbonate ions in the tubular urine into carbonic acid. This carbonic acid dehydrates to some extent to CO_2 and H_2O, and the CO_2 diffuses back across the tubular epithelium to enter the hydration cycle in the tubular cell. The sodium ions are reabsorbed along with equivalent numbers of bicarbonate ions. Distal reabsorption of bicarbonate like distal

sium in an outward direction. Chloride is reabsorbed in an amount sufficient to achieve ionic equivalence. It is possible that this reabsorption of chloride is passive, the ions moving downhill into the peritubular fluid along an electrochemical gradient in much the same fashion and driven by the same forces postulated in the proximal tubule. The movement of bicarbonate ions from the interior of the cell to the peritubular fluid may also be downhill along the existing potential gradient. A second pump, located in the peritubular membrane of the cell, ejects sodium from the cell, maintaining the intracellular sodium concentration at a low value. This pump like that of the proximal tubular cell originates the transcellular potential in some manner or other. It might be an electrogenic ion pump or it might be electrically neutral and transport potassium ions into the cell by the coupled carrier mechanism postulated earlier.

OPERATION OF ION EXCHANGE PROCESSES IN THE DISTAL NEPHRON

An appreciation of the operation of distal tubular ion exchange in the regulation of acid-base balance and potassium metabolism is necessary for an understanding of the mechanism of diuresis induced by acidifying salts, potassium salts, and carbonic anhydrase inhibitors, and for elucidation of the potassium deficit which may result from intensive therapy with any diuretic. Normal operation of the ion exchange mechanisms will be considered briefly in the following paragraphs; alterations in their operation in diuretic therapy will be discussed in Chapters XI, XVII, XVIII and XIX.

Evidence for Exchange of Hydrogen Ions for Sodium Ions in the Formation of Urinary Titratable Acid was first presented by Pitts and Alexander in 1945. They observed that dogs, rendered acidotic by the administration of ammonium chloride and infused with large quantities of neutral sodium phosphate, excrete far more titratable acid in the urine than is present in the glomerular filtrate. The renal tubules must add hydrogen ions to the tubular urine. Earlier Montgomery and Pierce had observed that the urine is acidified in the distal segment of the amphibian nephron, and

buffer content of the urine is low. Briggs, Pitts, Berliner and others have proposed that ammonia diffuses passively from tubular cells into tubular urine down a gradient of hydrogen ion concentration. Walker first showed in the amphibian kidney that ammonia is secreted into the tubular urine at the site of acidification, a finding confirmed by Pitts et al in the mammalian kidney by means of the "stop-flow" technique described in Chapters XVI and XVII. If as shown in Figure 15C, only salts of strong non-buffer acids are present in the urine in appreciable quantities, essentially no titratable acid can be excreted, for the tubules can develop a hydrogen ion gradient no greater than 1,000:1 between urine and blood (urine pH, 4.4; blood pH, 7.4). Accordingly little sodium could be salvaged by an ion exchange process which resulted only in formation of titratable acid, for under normal conditions, the amount of fixed buffer available to neutralize hydrogen ions in the urine is relatively small. However, ammonia diffuses from tubular cells into acid urine as un-ionized NH_3, buffers H^+ ions to form NH_4^+ ions, and permits the continued exchange of H^+ for Na^+ ions. Ordinarily ammonia buffers two to three times the quantity of hydrogen ions buffered by phosphate and anions of other weak acids.

According to Van Slyke, Archibald and their associates, distal tubular cells contain an enzyme glutaminase which catalyzes the hydrolysis of glutamine to glutamic acid and ammonia. Most of the urinary ammonia is derived from glutamine, delivered to the distal tubular cells in the peritubular blood. A minor source of urinary ammonia is amino acids, oxidatively deaminized to the corresponding keto acids and ammonia. Cell membranes are relatively permeable to un-ionized NH_3, impermeable to NH_4^+ ions. Ammonia therefore diffuses from its site of formation within tubular cells to lumen, where it is trapped as ammonium ion. The actual transport of ammonia is passive and dependent on a gradient of concentration across the luminal membrane of the cell.

Participation of Ion Exchange Mechanisms in Acid-Base Regulation. The three mechanisms described above participate in the regulation of acid-base balance in the following way. The usual mixed diet is acid ash; i.e., its content of fixed acid anions, chloride,

elaboration of titratable acid is depressed by carbonic anhydrase inhibitors. In fact the two mechanisms are one and the same. If little bicarbonate and much fixed buffer (phosphate) is present in distal tubular urine, the exchange of hydogen ions for sodium ions results mainly in the formation of titratable acid. If much

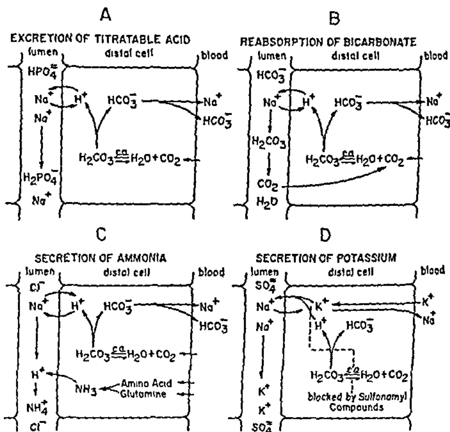


Fig. 15. The operation of the distal tubular and/or collecting duct ion exchange mechanism in the regulation of acid base balance and in the regulation of potassium content of extracellular fluid.

bicarbonate and little phosphate is present, the exchange of hydrogen ions for sodium ions results mainly in the reabsorption of bicarbonate.

Buffering of Hydrogen Ions by Ammonia permits continued exchange of hydrogen ions for sodium ions even though the fixed

exchanged for sodium ions, body stores of potassium are depleted and acidosis results.

Under normal conditions some 80 per cent of the filtered potassium is reabsorbed, only 20 per cent is excreted (see Table IV). Berliner believes that all of the filtered potassium is reabsorbed in the proximal tubule and that the moiety excreted is derived entirely from that secreted by the distal tubules in exchange for sodium. The evidence for this statement is by no means conclusive.

Ullrich and his associates have recently demonstrated by the ingenious technique of catheterizing the orifices of collecting ducts with filamentous polyethylene catheters that hydrogen-sodium exchange and ammonia secretion are functions of the collecting ducts as well as of the distal convoluted tubules. One should therefore describe these mechanisms as located in the distal nephron, not as previously, in the distal convoluted tubule. "Stop-flow" studies are consonant with this view.

Electron Microscopic Studies of Renal Tubules have demonstrated an amazing complexity and diversity of structure in the several segments of the nephron. Unfortunately, our understanding of both structure and function is at present too inadequate to permit the formulation of any very significant correlations between the two. However, to a physiologist, the following morphological features seem especially pertinent to an understanding of function.

First, the areas of the luminal and basal surfaces of tubular cells are tremendously increased by a complicated series of evaginations and invaginations of the limiting plasma membrane (see Fig. 16). In the proximal segment, the luminal surface of the cell is densely populated with cylindrical fingerlike evaginations, called microvilli, the brush border of light microscopists. These processes average 10,000 Å (1 micron) in length and 700 Å in diameter. They are covered with a thin plasma membrane and are otherwise structureless. In the conventionally fixed specimen, they are closely packed, some 215 per square micron, although in life with the tubule distended, they are no doubt separate and free floating in the tubular fluid. Tiny coiled ducts, which open at the

phosphate, and sulfate exceeds its content of fixed cations, sodium, potassium, calcium, and magnesium. Stated in another way, the normal diet imposes a fixed acid load on the body, a load which is neutralized by buffers of the body fluids, largely bicarbonate. If the acid anions were excreted as sodium salts, body reserves of buffer would be depleted and body fluids would become acid. Actually sodium is conserved, and the excess of anions is excreted either as titratable acid (mechanism A of Fig. 15) or in combination with ammonia (mechanism C of Fig. 15). The filtered sodium bicarbonate which escapes reabsorption in the proximal segment is completely removed from the distal urine (mechanism B of Fig. 15).

Exchange of Potassium Ions for Sodium Ions is involved in the regulation of acid-base balance and in the regulation of extracellular concentration and, indirectly, total body content of potassium. The exchange mechanism was first described by Berliner, Gilman and Mudge. Because they early recognized that hydrogen ions and potassium ions compete in exchange for sodium, the mechanism was assigned to the distal tubule. Confirmation of distal localization in the nephron of the dog has been provided by studies utilizing the "stop-flow" technique. Figure 15D illustrates the major characteristics of potassium exchange. If cellular and extracellular reserves of potassium are increased by the administration of potassium salts, potassium ions rather than hydrogen ions are exchanged for sodium. Failure to excrete hydrogen ions results in the development of hyperkalemic metabolic acidosis, usually of minor proportions. If cellular and extracellular reserves of potassium are depleted due to inadequate intake or excessive loss, hydrogen rather than potassium ions are exchanged for sodium. Enhanced excretion of hydrogen ions results in the development of hypokalemic metabolic alkalosis, a form of alkalosis resistant to treatment except by correction of the potassium deficit. Adrenal steroids enhance the overall activity of the exchange mechanism, thus increase both hydrogen and potassium exchange. Steroid excess therefore induces both hypokalemia and alkalosis. Inhibitors of carbonic anhydrase reduce the supply of hydrogen ions to the exchange mechanism. Potassium rather than hydrogen ions are

The basilar surfaces of both proximal and distal tubular cells are extensively infolded to form what are called cytoplasmic lamellae by Rhodin. These lamellae penetrate more or less deeply into the tubular cell (see Fig. 16) to divide its basal portion into a series of narrow open ended compartments in which mitochondria are linearly arranged. The basement membrane follows these invaginations, forming a double walled slit in open communication with the peritubular space. The slits are extensively branched and interconnected and tremendously increase the area of the basal surface of the cell. In the proximal segment and collecting ducts, the slits penetrate less deeply into the soma of the cells and the mitochondria are less obviously aligned with the surface invaginations than in distal cells. One might theorize that the arrangement in distal tubular cells would especially favor active pumping of ions. Mitochondria adjacent to the surface invaginations could supply phosphate bond energy to drive ion pumps in the membrane. Perhaps the alignment of mitochondria and slits is more evident in distal cells because of the greater energy expenditure demanded by the development of ion gradients in this segment.

A second feature of some significance is the delicate structure of peritubular capillaries. These vessels consist of a very thin fenestrated endothelial layer and a thin basement membrane. They are closely applied to the tubular cells. The system appears to be one which would permit the ready transfer of water and solutes between tubular cells and blood stream.

A third feature of note are the specializations to form a "tight tube." In proximal and distal tubules trabecular processes extend out radially from the basal portions of the cells. These trabeculae insert under adjacent cells and interlock with trabeculae of those cells. In the thin segment of the loop of Henle, where the cells are much attenuated, the cell borders are serrated and enmesh as a series of gear teeth. or more appropriately as pieces of a jig saw puzzle. Throughout the tubule, the chinks between cells are sealed at the luminal surface by terminal bars The arrangement would appear to be one to ensure that whatever crosses the tubular epithelium in either direction does so by transfer through the cell. Leakage between cells must be minimal. The correlations of

bases of the microvilli, penetrate into the soma of proximal cells. Some of these ducts connect with cytoplasmic vacuoles. Microvilli and microducts are most numerous in the convoluted portion of the proximal tubule. *They diminish in number in the thick descending* portion of Henle's loop, and are present only in rudimentary form



Fig. 16. Schematic representation of the structure of a proximal tubular cell as revealed by the electron microscope. (From J. Rhodin: Thesis. Stockholm, 1954, Karolinska Institute)

in the thin segment of the loop, distal tubule and collecting duct. These structures greatly increase the luminal surface available for diffusion, in fact for exchanges of any type between cell and tubular fluid. In view of the large quantities of water, sodium, chloride and hydrogen ions transferred across the luminal borders of proximal tubular cells, microvilli and microducts might be considered as rather simple but effective specializations increasing surface and therefore favoring bulk transfer.

The basilar surfaces of both proximal and distal tubular cells are extensively infolded to form what are called cytoplasmic lamellae by Rhodin. These lamellae penetrate more or less deeply into the tubular cell (see Fig. 16) to divide its basal portion into a series of narrow open ended compartments in which mitochondria are linearly arranged. The basement membrane follows these invaginations, forming a double walled slit in open communication with the peritubular space. The slits are extensively branched and interconnected and tremendously increase the area of the basal surface of the cell. In the proximal segment and collecting ducts, the slits penetrate less deeply into the soma of the cells and the mitochondria are less obviously aligned with the surface invaginations than in distal cells. One might theorize that the arrangement in distal tubular cells would especially favor active pumping of ions. Mitochondria adjacent to the surface invaginations could supply phosphate bond energy to drive ion pumps in the membrane. Perhaps the alignment of mitochondria and slits is more evident in distal cells because of the greater energy expenditure demanded by the development of ion gradients in this segment.

A second feature of some significance is the delicate structure of peritubular capillaries These vessels consist of a very thin fenestrated endothelial layer and a thin basement membrane. They are closely applied to the tubular cells. The system appears to be one which would permit the ready transfer of water and solutes between tubular cells and blood stream.

A third feature of note are the specializations to form a "tight tube." In proximal and distal tubules trabecular processes extend out radially from the basal portions of the cells. These trabeculae insert under adjacent cells and interlock with trabeculae of those cells. In the thin segment of the loop of Henle, where the cells are much attenuated, the cell borders are serrated and enmesh as a series of gear teeth. or more appropriately as pieces of a jig saw puzzle. Throughout the tubule, the chinks between cells are sealed at the luminal surface by terminal bars. The arrangement would appear to be one to ensure that whatever crosses the tubular epithelium in either direction does so by transfer through the cell. Leakage between cells must be minimal. The correlations of

structure and function outlined above must be considered only as speculations.

SUMMARY

Urine formation begins with the ultrafiltration of large volumes of plasma through the porous walls of the glomerular capillary tufts. The glomerular ultrafiltrate contains all crystalloidal components in essentially the same concentrations as exist in the aqueous phase of the plasma from which it is formed. To prevent rapid exhaustion of limited body reserves, the bulk of the filtered water and of the filtered sodium, chloride, and bicarbonate ions is reabsorbed by the renal tubules; less than 1 per cent is normally excreted. It is possible to account for a number of these reabsorptive functions in terms of the active tubular transport of a single ion species, sodium.

It is probable that sodium pumps, located in the peritubular membrane of proximal tubular cells, account for the active reabsorption of the major fraction (4/5ths to 7/8ths) of the filtered sodium. The electrical forces set up by such pumps may cause the passive migration of chloride ions into the blood stream along a favorable electrical gradient. Bicarbonate ions may be reabsorbed indirectly in the proximal segment by the passive migration of hydrogen ions from tubular cell to tubular lumen. The osmotic force created by the active reabsorption of ions and other solutes accounts for the passive transfer of an equivalent proportion of the water.

The conditions governing ion reabsorption in the loops of Henle, in the distal tubules, and in the collecting ducts are more demanding. Concentration gradients developed for sodium and hydrogen ions and the interrelations of sodium, hydrogen and potassium transport suggest that the reabsorptive mechanisms in the distal parts of the nephron are more complicated. Perhaps two types of sodium pumps are necessary, one type located in the luminal membrane, the other in the peritubular membrane. Both may be coupled ion pumps.

The elaboration of urine hypertonic to the blood plasma, a function now generally conceded to reside in the collecting ducts, need not involve the active transport of water. The active

pumping of sodium from the ascending limbs of the loops of Henle into the interstitium of the medulla and papilla renders the tissue hypertonic. Water moves from the collecting ducts into the interstitium by osmosis; the osmolal concentration of the final urine is essentially the same as that of papillary tissue.

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Chapter V

REGULATION OF VOLUME AND OSMOLAL CONCENTRATION OF EXTRACELLULAR FLUID

THE volume of extracellular fluid, as reflected in the body weight of a normal adult in caloric balance, is held remarkably constant from day to day despite variations in intake of salt and water. The osmolality of extracellular fluid is stablized with even greater precision. Within limits, volume and osmolality are regulated *independently; however under stress, the regulatory* mechanisms interact, and precise regulation of one variable may be sacrificed to permit some control of the other. A volume receptor-renal effector mechanism governs the rate of excretion of salt, thus the total salt content and hence the volume of the extracellular compartment. Actually the complete regulatory mechanism must involve salt appetite as well, but since the average diet contains more than enough electrolyte to satisfy needs, one ordinarily overlooks this element of the system. An osmoreceptor-renal effector mechanism governs the rate of excretion of water, and in association with thirst, regulates the osmolality of the extracellular fluid.

REGULATION OF EXTRACELLULAR VOLUME

The Volume Receptor-renal Effector Complex is no doubt a natriuretic-antinatriuretic mechanism; i.e. it basically regulates the excretion of sodium. While affected by, it is not primarily responsive to the concentration of sodium. Rather it is responsive to the volume of extracellular fluid; to some fraction of that volume, such as that of plasma or interstitial fluid, to some derivative of volume, such as intra-vascular or interstitial pressure; or perhaps to blood flow. It is probable that the system consists of a

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tonicity of medullary and papillary tissue, thus with the elaboration of concentrated urine; and a distal tubule-collecting duct mechanism, concerned with acid base regulation and potassium excretion, with the elaboration of salt-poor dilute urine, and with the fine regulation of sodium balance.

The rate at which sodium is reabsorbed by the proximal mechanism varies as some function of glomerular filtration rate, but to date it has been impossible to describe the function precisely. According to Walker and his associates, some 12.5 per cent of the fraction of sodium remaining in the proximal segment is reabsorbed in each succeeding 10 per cent of tubule length. According to Smith, roughly seven-eights of the filtered sodium is reabsorbed at all levels of filtration rate. Suffice it to say that if filtration rate increases, the absolute amount of sodium reabsorbed in the proximal segment increases; if filtration rate decreases, the absolute amount of sodium reabsorbed decreases. However, of more immediate significance for the maintenance of sodium balance is the fact that an increase in filtration rate results in the delivery of more sodium into distal parts of the nephron; a decrease in filtration rate results in the delivery of less sodium. If, as seems probable, the reabsorptive capacities of these distal segments are limited, the rate of excretion of sodium will vary directly as some function of filtration rate.

The mechanism of the loop of Henle probably reabsorbs at least 5 per cent of the filtered sodium. This statement derives from the fact that some 4 to 5 ml. of solute free water are reabsorbed in the collecting ducts in the final transformation of isotonic tubular fluid into maximally concentrated urine. The motive force for this reabsorption of water is the hyperosmolality of medullary and papillary tissue created by the reabsorption of sodium in the loops of Henle. The quantity of water which can be reabsorbed in the collecting ducts is obviously related to the quantity of sodium reabsorbed by the ascending limbs of the loops of Henle.

Under normal conditions the distal tubule-collecting duct mechanism reabsorbs some 10 to 15 per cent of the filtered sodium. This mechanism is a heterogeneous one, engaged in the reabsorption of sodium and chloride as ion pairs and in the reabsorption of

receptor organ which senses volume, pressure, or flow, a hypothalamic integrative center, and an efferent neuro-humoral regulatory mechanism which operates through the kidney to control the rate of excretion of salt. In no wise is any part of this system as clearly and adequately defined as is the corresponding part of the osmo-regulatory mechanism. Much of what can be said must be considered as speculative.

Volume Receptor Organs. As one might gather from the statements above, both the nature of the stimulus and the locus and nature of the receptor endings are in dispute. Harrison and Strauss maintain that the receptor mechanism is in the cephalad portion of the body and that it is either directly sensitive to the extracellular volume or to its derivatives, venous pressure or distension. Epstein on the other hand suggests that the receptor mechanism is in the arterial reservoir and that the degree of distension of this reservoir generally, or the distension of some highly sensitive portion of it, initiates the afferent messages which ultimately modulate salt excretion. Borst, in contrast, believes that the receptor mechanism is sensitive to variations in cardiac output or perhaps to cardiac output relative to metabolic demands of the body.

Integrative Mechanism. The locus of the integrative mechanism is unknown, but it is logical to assume that it is in the hypothalamus for the following reasons. Although afferent impulses for the most part are received from volume receptors of unknown location, efferent outflow of the integrative center is directed to hypothalamic and cortical centers for thirst and salt craving; to hypothalamic autonomic centers controlling renal hemodynamics, to a postulated diencephalic center controlling aldosterone secretion, and to the hypothalamic center for osmoregulation. It is likely that the integrative center which serves to coordinate the activities of these several mechanisms is also in the hypothalamus.

Renal Effector Mechanism. Mechanisms of reabsorption of sodium have been described in Chapter IV. Broadly speaking, they fall into three categories: A proximal mechanism, concerned with the reabsorption of the bulk of the filtered sodium; a mechanism in the loop of Henle, concerned with the maintenance of hyper-

constancy of extracellular fluid volume. (1) If salt intake increases, extracellular volume expands; volume receptors are stimulated. Nerve mesasges relayed through the integrative center, a hypothalamic autonomic center and renal vasomotor nerves, increase renal blood flow and glomerular filtration rate. More sodium is delivered to the reabsorptive mechanism of the distal tubules and collecting ducts; more escapes reabsorption and is excreted. The inverse sequence, initiated by a decrease in extracellular volume, leads to reduced filtration, to over-reabsorption, and to diminished excretion of sodium. As will be evident below, this mechanism plays a prominent role in the dog. (2) Expansion of extracellular volume leads via the integrative center and the diencephalic center regulating aldosterone secretion, to reduced secretion of salt retaining steroid. Reabsorption of sodium in the distal tubules and collecting ducts diminishes and excretion increases. The inverse sequence, initiated by a decrease in extracellular volume, results in increased reabsorption and diminished excretion of sodium. As will be evident below, this mechanism plays a prominent role in man.

Response to Salt Loading. Normal man tolerates variations in salt intake within a range of 1 to 10 gm. per day with minimal fluctuations in body weight. However, if some 30 or 40 gm. of salt are added to the diet each day, body weight increases promptly and stabilizes at a value some 5 to 15 lb. above normal. This increase in body weight is due to expansion of extracellular fluid volume, i.e., to the collection of occult and even overt edema. As Luetscher, Bartter and others have shown, the rate of urinary excretion of salt retaining steroids is markedly diminished or indeed abolished under such conditions. Stabilization of the volume of extracellular fluid at a moderately elevated level instead of progressive sodium retention is no doubt made possible largely by abolition of aldosterone stimulation of a small but highly significant fraction of salt reabsorption. According to Leaf *et al.*, Green *et al.*, and Crawford and Ludemann, salt loading causes relatively little increase in glomerular filtration rate in normal man. This no doubt underlies his relative intolerance of high dietary salt intake and his very sluggish excretion of an intravenous load of saline.

sodium in exchange for hydrogen, ammonia, and potassium. Overall reabsorptive capacity is limited. Hence if the mechanism is presented with an excess of sodium in consequence of increased filtration rate, reabsorptive capacity is exceeded and excretion increases; if presented with less sodium, all may be reabsorbed. The transport capacity of the distal tubule-collecting duct mechanism is enhanced by aldosterone, but the steriod sensitive fraction of reabsorption is probably small. This latter statement derives from the fact that the adrenalectomized dog or man excretes only 2 per cent or so of the filtered sodium (see Chapter VI). It is possible that the aldosterone sensitive fraction of reabsorption is greater than 2 per cent, but that in the absence of hormone, other regulatory mechanisms compensate for most of the reabsorptive deficit. In any event, hormonal control of a final, albeit small, fraction of tubular reabsorption provides powerful leverage in the control of sodium balance.

If a small fraction of sodium reabsorption fails due to lack of circulating hormone (adrenalectomy, Addison's disease), body stores of sodium, chloride, and water are gradually depleted. Furthermore, because of reduced exchange of hydrogen, potassium and ammonia for sodium, hyperkalemia and metabolic acidosis develop. In contrast, when excessive amounts of aldosterone are secreted, hydrogen, ammonia, and potassium are exchanged for sodium in increased amounts. Hypokalemia and metabolic alkalosis develop and sodium, chloride, and water are retained. However the relationship is not a simple one. Primary hyperaldosteronism, due to glandular hyperplasia or adenoma, is characterized more by hypokalemia and alkalosis than by sodium retention. In contrast, secondary hyperaldosteronism, now recognized as occurring in variable degree in all states of active accumulation of edema, is characterized more by sodium retention than by hypokalemia and alkalosis. Other unrecognized factors must be significent in determining ion balances.

Mode of Action of Neurohumoral Regulatory Mechanisms. Given a series of reabsorptive mechanisms with the diverse properties described above, it is reasonable to assume that sodium output may be balanced against intake in two ways to achieve

it must contribute to glomerulo-tubular imbalance and to salt retention (see Chapter VI on renal factors in the formation of edema). However, a reduction in glomerular filtration rate is by no means the whole story. Goodyer and Jaeger have proposed an interesting hypothesis in explanation of immediate reduction of salt excretion on erect standing in subjects exhibiting no gross change in filtration rate. They propose that erect standing favors the formation of an increased proportion of filtrate in long, high salt absorbing nephrons and of a decreased proportion of filtrate in short salt wasting nephrons Unfortunately, there is no evidence in favor of this thesis other than the well documented observation of salt and water retention under conditions which lead to general compensatory alterations in hemodynamics. Most investigators have preferred to emphasize increased tubular reabsorption of salt and water, stimulated respectively by increased secretion of aldosterone and of antidiuretic hormone. In favor of this thesis is the observation of Muller and others of a diurnal variation in rate of excretion of aldosterone in the urine of normal man: high during the day (orthostasia), low at night (recumbency). Also favorable is the finding of Davis, Farrell and others that the rate of liberation of aldosterone into adrenal venous blood of the dog increases progressively during hemorrhage.

Two facts make it difficult to explain compensatory salt retention in man entirely in terms of increased secretion of aldosterone. First, retention of salt in response to erect standing, bleeding, or trapping of blood in the limbs is prompt; adrenal steroids given intravenously stimulate salt reabsorption after a latent period of 40 min. to an hour or more. Second, Rosenbaum et al have shown that patients with Addison's disease, on maintenance doses of adrenal steroids, exhibit a normal renal salt conserving response to bleeding, assumption of erect posture and compression of the thighs.

It seems evident that no single mechanism can explain salt retention under all circumstances of depletion of extracellular volume. Relative over-reabsorption from a reduced volume of filtrate, redistribution of filtrate among nephrons of diverse function, oversecretion of aldosterone and perhaps other as yet

In contrast the volume receptor-renal effector mechanism of the dog responds briskly and effectively to salt loading. Ladd and Raiz have shown that the dog can tolerate as much as 4 gm. of salt per Kg. per day for long periods of time, gaining weight with water ingestion after each meal, losing it overnight. Per Kg. of body weight, this quantity of salt would correspond to a load of 280 to 300 gm. per day in man, a value far in excess of any that can be tolerated. Green and Faragh, Wesson and Anslow, Mudge and others have shown that the rate of glomerular filtration of the dog increases promptly on salt loading by as much as 100 per cent and that, for brief periods, the rate of excretion of sodium may attain the phenomenal value of 40 per cent of that filtered. It is obvious that normal man, but not the normal dog, is only a "salt shaker" away from incipient edema, and that stability of filtration rate in man at least partially explains his intolerance of salt loading in comparison with the dog. It is understandable why even mild reduction of glomerular filtration rate or even moderate elevation of aldosterone production in cardiac, hepatic or renal disease may lead to manifest edema. Just why the dog should be so thoroughly protected against the stress of salt loading and man should be so vulnerable, is a mystery.

Response to Volume Depletion. While the homeostatic response of normal man to expansion of extracellular volume compares unfavorably with that of the dog, his response to the more immediately vital threat of volume reduction is prompt and effective. Goodyer, Seldin, Brun, Farber, Epstein and others have shown that urine flow and salt excretion drop precipitously when a relatively small proportion of the circulating blood volume is sequestered by venous tourniquets on the thighs, is redistributed by erect standing or by opening an arterio-venous fistula, or is removed by phlebotomy. All three procedures excite the sensation of thirst. While the response is obviously of homeostatic significance in combating an actual or apparent reduction of extracellular volume, the responsible mechanisms have by no means been adequately defined. Glomerular filtration rate has been variously described as decreasing moderately, decreasing equivocally, and exhibiting no change. If filtration rate decreases, it is evident that

It is apparent from recent evidence that ACTH does exert some influence over aldosterone production, injection of ACTH increasing, hypophysectomy decreasing glandular secretion of salt active materials. However, the effects of ACTH or hypophysectomy on aldosterone production are far less marked than on glucocorticoid production. The basic conclusions drawn from the work of Deane and Greep are still valid.

Farrell and his associates have recently observed that decorticate, but not decerebrate or decapitate animals secrete normal quantities of aldosterone into adrenal venous blood, an observation which suggests that a diencephalic neurosecretory mechanism, independent of the hypophysis, controls hormone production. In support of this view, they have observed that injection of extracts prepared from the diencephalon causes the discharge of aldosterone into the adrenal veins. At a recent Lauretian conference they reported that an acid extract of the subpineal region of the posterior diencephalon is most active in stimulating aldosterone secretion. They draw an analogy between the neurosecretory mechanism of the supraopticohypophyseal system which controls the renal reabsorption of water and that of the diencephalic-adrenal system which controls the renal reabsorption of salt. If their observations are confirmed, the nature of the neurohumoral control of aldosterone secretion will have been considerably advanced.

Interaction of Volume and Osmoregulatory Mechanisms. Patients in congestive failure who are subjected to vigorous diuretic therapy or those with cirrhosis from whom large volumes of ascitic fluid are removed by paracentesis may respond to such acute reductions of extracellular or transcellular fluid volume by vigorous retention of both salt and water. They experience marked thirst, and if water intake is unrestricted, may dilute their body fluids to a degree sufficient to produce signs and symptoms of severe water intoxication. They do not exhibit a normal water diuresis although their body fluids are markedly hypotonic. A somewhat similar syndrome occurs in otherwise normal individuals who sweat profusely. Sweating depletes the body of both salt and water and reduces extracellular volume. Replacement of water causes dilution, muscular cramping, and weakness. This syndrome suggests that

unrecognized mechanisms may contribute to the response. The difficulty of assessing the significance of small changes in filtration rate versus small changes in tubular reabsorptive activity in explanation of the retention of salt should be apparent to anyone who reflects for a moment on the facts that the total daily variation in excretion is commonly less than ± 170 mEq. of sodium; that the total filtered load is roughly 24,000 mEq., that the change in reabsorption is from 99 to 99.9 per cent of the filtered load; and that reabsorption is effected, not by a single mechanism, but by a variety of mechanisms of diverse properties distributed serially along the nephron. The final difficulty is that the measurement of filtered load is at best uncertain to ± 2 per cent.

Site and Nature of Neuro-humoral Regulatory Mechanisms. During the latter part of the last Century, piqure of the bulb, thalamus and hypothalamus were variously described as producing urinary salt wasting and hypochloremia, or urinary salt retention and hyperchloremia. Welt and his associates have more recently described a series of patients with diffuse central nervous system disease exhibiting a salt wasting syndrome, associated with low serum sodium and chloride. The evidence suggests that the central nervous system is in some way involved in the renal regulation of electrolyte balance, *yet it clarifies neither the locus nor the mode* of action of the neural structures involved.

It has long been known that the hypophysectomized animal or the patient with pan-hypopituitarism exhibits gross evidence of multiple endocrine deficiencies, including those of glucocorticoid deprivation. Equally clear has been the fact that the hypopituitary organism does not suffer from the severe electrolyte disturbances which are associated with adrenal insufficiency. The observation of Deane and Greep that the zona glomerulosa of the adrenal of the hypophysectomized animal retains its integrity, suggests that aldosterone is formed in this portion of the adrenal cortex, that the functional integrity of the aldosterone producing cells is independent of hypophyseal trophic hormones, and that the rate of secretion of aldosterone is regulated in accord with the needs of the body by some hormone other than adrenocorticotrophic hormone (ACTH).

the osmolal concentration falls. Shrinkage is thought to stimulate the processes of neurons of the supraoptic nucleus applied to the surfaces of the vesicles. Stimulation of these neurons leads to the secretion of antidiuretic hormone. Inhibition of these neurons by swelling of the vesicles stops hormone production and that which circulates is gradually destroyed by the tissues.

The injection of hypertonic solutions of sodium chloride into exteriorized carotid artery loops of trained dogs results in the prompt inhibition of water diuresis. The effect is produced by a small dose, calculated to increase the osmolality of the carotid blood by only two per cent. The injection of a much larger dose into a peripheral vein where it is diluted in a large volume of blood before reaching the receptor area has no effect on diuresis. The injection of urea into the carotid loop, a substance to which the vesicles are apparently freely permeable, has no effect on water diuresis; i.e., it exerts no osmotic effect to cause shrinkage of osmoreceptor vesicles.

Hypothalamic Integrative Mechanism. While the osmolal concentration of the blood plasma acting through osmoreceptors in the brain basically regulates the secretion of antidiuretic hormone, a variety of agents and stimuli exerts a subsidiary control. Antidiuresis due to liberation of antidiuretic hormone results from painful stimuli, exercise, syncope, smoking and from the administration of such drugs as nicotine, adenosine tri-phosphate, acetylcholine, epinephrine and histamine. Anesthetics, especially ether, morphine and the barbiturates are potent stimulators of antidiuretic hormone secretion. On the other hand, the release of antidiuretic hormone is supressed and diuresis results when the left atrium is distended by a balloon, when the suggestion of water drinking is made under hypnosis, as a result of establishment of conditioned reflexes and from the administration of alcohol These several excitatory and inhibitory stimuli are presumably integrated in a hypothalamic center, but whether in the supraoptic or paraventricular nuclei or in the adjacent hypothalamic tegmentum is unknown.

Neurosecretory Mechanism. The view that the antidiuretic hormone is actually formed in the cells of the supraoptic and para-

the volume regulatory center exerts an ancillary control over the osmoregulatory mechanism. If volume is markedly reduced, the antidiuretic mechanism is maximally engaged, even though the body fluids are excessively diluted. Volume is partially restored at the expense of a reduction in osmolality.

REGULATION OF OSMOLAL CONCENTRATION OF BODY FLUIDS

Given free access to water and a diet of even minimal salt content, the normal individual regulates the osmolal concentration of his body fluids with remarkable precision. Normal limits are usually given as 285 and 310 mOsm. per liter. However, any one individual exhibits even greater constancy, varying only one or *two per cent from his characteristic mean. Osmolality is regulated* by the variable excretion of water in relation to osmotically active solutes. When the body is diluted by the ingestion of large quantities of water, diuresis of hypotonic urine restores the osmolal concentration of the body fluids to normal. When the body is concentrated by loss of water or by gain of solute, oliguria restricts further water loss; the formation of hypertonic urine permits the excretion of solute without the loss of equivalent amounts of water; and thirst drives the individual to restore his water deficit. The regulatory mechanism consists of an osmoreceptor system, a hypothalamic integrative mechanism, a neurosecretory mechanism producing antidiuretic hormone and a renal effector mechanism which governs the excretion of water. In contrast to the sluggish regulation of volume when an individual is salt loaded, osmolal concentration is promptly restored to normal when he is water loaded.

Osmoreceptor Mechanism. The elegant studies of Verney have demonstrated that receptors, sensitive to small changes in osmolality of the blood plasma perfusing them, are located bilaterally within the zones of distribution of the internal carotid arteries. He has suggested that small vesicles in the supraoptic nuclei of the hypothalamus may be the osmoreceptors. These vesicles are presumed to act as minute osmometers, to shrink when the osmolal concentration of their fluid environment rises, and to swell when

quarter hour thereafter takes an amount equivalent to the volume of urine excreted, he achieves and maintains a maximal state of hydration. Urine flow increases within an hour to 13 to 26 ml, per min., i.e. to a level which is maximal for that individual and which cannot be increased by more rigorous hydration procedures. If more water is ingested, it usually leads to nausea and vomiting or to diarrhea, not as might be presumed to water intoxication, to which the normal adult is remarkably resistant. The child or the patient with renal, hepatic, adrenal, or cardiac disease is considerably less tolerant of water and exhibits water intoxication more readily.

Hydration of this magnitude dilutes the body and reduces the osmolality of the body fluids by 3 to 5 per cent. The secretion of antidiuretic hormone is suppressed, that hormone which normally circulates is metabolized, and a state of physiological diabetes insipidus develops. A polyuria of dilute urine results. Nearly pure water is excreted in defense of the osmotic pressure of the body fluids.

When water intake is terminated, urine flow remains high for a time and the excess water in the body is eliminated. As the osmolality of the body fluids approaches its normal level, osmoreceptors are again stimulated, antidiuretic hormone secretion begins, and urine flow is gradually reduced to more normal values.

If fluid is withheld for a period of time, the osmolality of the body fluids rises as water is lost by the insensible routes of cutaneous and pulmonary evaporation and by urine formation. The osmoreceptors are stimulated to a greater than normal degree and antidiuretic hormone secretion increases above its basal rate Urine flow is reduced from its usual value of 1 to 2 ml. per min. to 0.5 ml. or less. The urine is highly concentrated, the solutes ordinarily excreted in 2 ml. of urine are now contained in 0.5 ml. or less. The osmolal concentration of the urine may increase to a value some 4 to 5 times that of the plasma. Obviously for each 0.5 ml. of urine excreted with an osmotic pressure 4 times that of plasma, the body gains 1.5 ml. of free water to expend in insensible evaporation or to dilute the body fluids. However, in a quantitative sense, the osmoreceptor-renal effector mechanism is far more

ventricular nuclei and transported to the posterior lobe of the pituitary by protoplasmic flow in axons of the supraoptico-hypophyseal tracts was first enunciated by Sharrer a number of years ago. The material formed in the neurons, i.e. the neurosecretory material, is thought to be a protein of molecular weight of 30,000 to which is bound one molecule of antidiuretic hormone and one of oxytocin. The pituicytes of the posterior lobe of the pituitary, once considered to be the actual secreting cells, are now believed to play some role in the release of active hormone fragments from the neurosecretory protein in the nerve terminations.

Nature of the Antidiuretic Hormone. DuVigneaud has determined the structure and synthesized two antidiuretic hormones derived respectively from beef and hog pituitaries. Arginine-vasopressin is derived from beef pituitaries and probably occurs in the glands of man, monkey, dog, rat, sheep and camel. Lysine-vasopressin is derived from hog pituitaries.

CYS - TYR - PHE - GLU(NH_2) - ASP(NH_2) - CYS - PRO - ARG - GLY(NH_2)

Arginine-Vasopressin (Beef)

CYS - TYR - PHE - GLU(NH_2) - ASP(NH_2) - CYS - PRO - LYS - GLY(NH_2)

Lysine-Vasopressin (Hog)

Commercial pitressin is a mixture of the two. The hormones can be considered as octapeptides consisting of a 5-membered ring made up of tyrosine, phenyl alanine, glutamine, asparagine and cystine and a 3-membered side chain of proline, either arginine or lysine, and glycinamide. In the structures shown above, two molecules of cysteine are joined in disulfide linkage to form a closed ring; i.e., a ring containing one molecule of cystine.

The term vasopressin is strictly a misnomer and derives from the common method of assay of commercial preparations in terms of their potency in elevating blood pressure of the experimental animal. The hormone is many orders of magnitude more active in its antidiuretic action on the kidney than as a pressor agent. It is probably never secreted in amounts necessary to raise blood pressure significantly.

Role of Antidiuretic Hormone in the Control of Osmolality. If an individual ingests rapidly two liters of water and every

U/P ratio is a more sensitive indicator of water conservation than is urine flow when flow is very low. It is apparent that the infusion of 7.5 milliunits of pitressin per hr. decreased urine flow of a normal man from 16 ml. per min. to 1.4 ml. per min. and increased creatinine U/P ratio from 7 to 80. Increasing the rate of pitression infusion first to 18 and then to 50 milliunits per hr. further depressed urine flow from 1.4 to 0.6 ml. per min. and increased creatinine U/P ratio from 80 to 180. The renal response to the infusion of 50 milliunits of pitressin per hr. was the greatest that could be attained, and is comparable to that observed in moderate dehydration. It is, therefore, reasonable to assume that the dehydrated subject produces antidiuretic hormone at essentially this rate. According to Lauson, normal man regulates urine flow over the physiological range by variably secreting antidiuretic hormone at rates of 0.1 to 0.8 milliunits per hr. per Kg. body weight. Shannon and Verney have found comparable rates of secretion in the dog.

Site and Cellular Mechanism of Action of Antidiuretic Hormone. In Chapter IV it was pointed out that antidiuretic hormone controls the permeability of the distal tubules and collecting ducts to water. Under conditions of maximum hydration, i.e. in the absence of circulating ADH, these structures are impermeable to water. Continued reabsorption of sodium results in the excretion of a large volume (15 to 20 ml. per min.) of hypotonic urine. Under conditions of hydropenia, i.e., in the presence of a high titre of circulating ADH, both distal tubules and collecting ducts are freely permeable to water. Hypotonic fluid, entering the distal tubules from the loops of Henle, loses water to and equilibrates osmotically with blood in the cortical capillaries. Continued reabsorption of sodium, coupled with free transfer of water, reduces volume at the end of the distal tubules to 3 to 6 ml. per min. This fluid is isosmotic with systemic blood. In the collecting ducts water is lost to the hypertonic medullary and papillary tissue. The urine is reduced in volume and becomes as hypertonic to systemic blood as the tissue at the tip of the papilla.

Koefoed-Johnsen and Ussing observed that antidiuretic hormone greatly increases the permeability of isolated frog skin to water. Their analysis led them to believe that the skin is penetrated

effective in defending the body against dilution than against dehydration. Ultimately thirst must drive the individual to replace water deficits; the kidney cannot replace them.

Rate of Antidiuretic Hormone Secretion. The condition of maximum sustained hydration described above is a favorable one in which to assess experimentally the probable range of normal rate of secretion of antidiuretic hormone. Verney and Shannon have studied this problem in the dog, Lauson has done so in man. If, when urine flow has stabilized at the high rate of 15 to 20 ml.

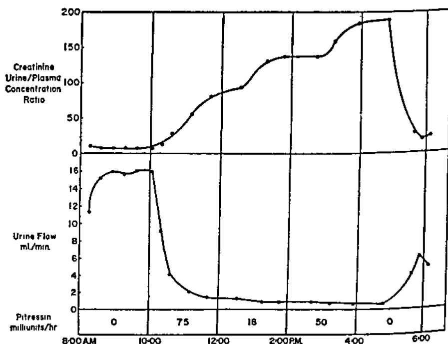


Fig 17. Effects of Pitressin in increasing amounts on creatinine urine plasma concentration ratio and urine flow of normal man. (Redrawn from data of H.D Lauson *Am. J. Med*, 11·135, 1951.)

per min., antidiuretic hormone is infused intravenously in low dosage, urine flow falls and the concentration of various urinary constituents increases. The degree to which creatinine is concentrated in the urine relative to the plasma, i.e, the creatinine U/P ratio, is a useful measure of the avidity with which the kidneys conserve water. As is apparent in Figure 17, the creatinine

supraoptic and paraventricular nuclei or in the adjacent hypothalamic tegmentum; a neurosecretory system which releases the hormone pitressin in amounts related to the need for conserving water; and a renal effector mechanism which responds to the titre of circulating hormone by varying urine flow. In the hydrated individual, pitressin secretion is inhibited, that which circulates is metabolized, and the distal tubules and collecting ducts become impermeable to water. A large volume of dilute urine is excreted. In the dehydrated individual, pitressin secretion is stimulated, the distal tubules and collecting ducts become permeable to water, and water reabsorption is enhanced. A small volume of concentrated urine is formed.

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by minute channels or pores, through which water flows when solutions of different osmolal concentrations are in contact with the two surfaces. In the absence of antidiuretic hormone these pores are small and the transfer of water is slow. In the presence of antidiuretic hormone the pores are greatly dilated and the osmotic transfer of water is rapid. Sawyer, Berliner and Gottschalk have suggested that antidiuretic hormone may exert its entire action on the renal tubule in a similar fashion, namely by dilating aqueous channels in cells of the distal tubules and collecting ducts, thus permitting osmotic equilibration of tubular contents and peritubular fluid.

SUMMARY

The volume of the extracellular fluid compartment is determined by its sodium content, the osmolal concentration, by its water content relative to sodium. Although volume and osmolality are interrelated, they are separately regulated by mechanisms which are at least semi-independent.

The volume regulatory mechanism consists of receptors, which sense either volume or some derivative of volume such as pressure or flow; an integrative center, probably located in the hypothalamus; and a neurohumoral effector mechanism which controls the renal excretion of sodium. In health, sodium excretion precisely balances intake; accordingly, the sodium content and the volume of the extracellular compartment are held constant within narrow ranges of normal. The rate of excretion of sodium is dependent both on the rate at which sodium is delivered into the renal tubules in the glomerular filtrate and on the capacity of the tubules to *reabsorb sodium from the urine. Control of excretion is effected* by vasomotor nerves and by humoral agents which regulate glomerular filtration rate and by the secretion of aldosterone which regulates tubular reabsorptive capacity. Other less adequately defined mechanisms may also affect the excretion of sodium.

The mechanism regulating osmotic pressure consists of receptors, located within the zone of distribution of the internal carotid arteries and sensitive to changes in osmolality of the arterial blood of the order of ± 2 per cent; an integrative center, either in the

shown on the left, the extracellular fluid volume of a normal 70 Kg. man is roughly 14 liters. Enough salt and water are delivered into this volume by way of the gastrointestinal tract to cause it to expand at a rate of one-half to one or more liters per day. However, the normal individual is able to adjust the output of salt and water to the intake and thereby to maintain constancy of extracellular fluid volume. The edematous patient, in contrast, exhibits a relative incapacity to excrete salt and water and as a consequence, extracellular fluid volume expands. If, as shown in the diagram on the right, intake is markedly reduced, it may be brought into line with excretory capacity, and fluid volume may be maintained within normal limits, even though the basic and precipitating disease process persists. In this chapter, present concepts of the factors which cause this relative incapacity to eliminate salt and water will be outlined briefly.

Glomerulo-tubular Balance. It is evident from the discussion in the preceding chapters that the maintenance of sodium balance involves the failure to reabsorb a minute fraction of the total filtered load. It is, therefore, difficult to determine in any specific instance whether retention of sodium results from a deficiency in the filtered load or from enhanced tubular reabsorption of that load. One can grossly define the problem of maintenance of salt and water balance, and the nature of the disturbances which lead to accumulation of edema in terms of glomerulo-tubular balance. This concept is illustrated in Figure 19. The normal individual filters a normal amount of salt and water through his glomeruli, reabsorbs a normal amount, and hence excretes a normal amount. He, therefore, exhibits glomerulo-tubular balance. The quantity of salt and water excreted is usually 1 per cent or less of that filtered, accordingly, 99 per cent or more is reabsorbed.

If filtration rate is reduced and if there is no corresponding reduction in tubular reabsorptive activity, excretion decreases as indicated in the middle diagram. Conversely, if filtration rate is normal and if tubular reabsorptive activity is increased, as shown on the right, excretion likewise decreases. The small glomerulus in the middle diagram and the heavier tubule on the right are drawn to represent alterations in function, not morphologic changes. The

Chapter VI

RENAL FACTORS IN EDEMA FORMATION

EDEMA is a clinical sign, a manifestation of diseases of varied origin, not a disease entity in its own right. There are common features in the pathogenesis of edema in condition as varied as congestive heart failure, cirrhosis, nephrosis, nephritis, preeclampsia, protein starvation, and even thrombophlebitis; there are obviously differences as well. In this chapter, the common features will be emphasized. Furthermore, the edema of congestive failure

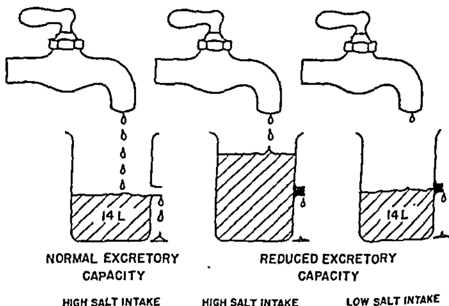


Fig. 18. Salt and water intake and excretory capacity in relation to edema

will receive primary consideration, for the problem is not only the most common but has also been most intensively studied.

The Role of the Kidneys in the Retention of Salt and Water in Edema is illustrated in diagrammatic fashion in Figure 18. As

some 35 per cent of the sodium contained in an intravenous load of 500 ml. of isotonic saline in the first hour and about 80 per cent in 8 hours. Tricuspid insufficiency alone reduced salt tolerance considerably, i.e., it reduced excretion during the first hour markedly, and total 8 hour excretion appreciably. These animals exhibited little or no additional evidence of circulatory inadequacy. The combined lesions of pulmonary stenosis and tricuspid insufficiency, bringing out the full blown syndrome of congestive right heart failure, reduced excretion of the sodium load to insignificant proportions, both at 1 and at 8 hours. These results are reminiscent of those of Schroeder, Burch and others who have shown that patients in congestive failure excrete a sodium load less readily than do normal individuals and that tolerance to sodium loading diminishes with increasing signs of congestion.

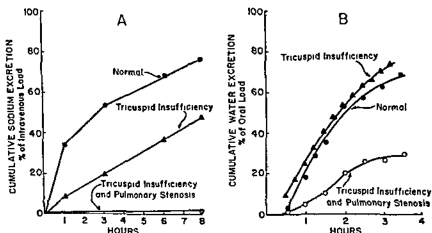


Fig 20. Effects of production of tricuspid insufficiency and tricuspid insufficiency plus pulmonary stenosis (chronic congestive heart failure) in the dog, A, on the excretion of an intravenous load of 500 ml of isotonic saline, B, on the excretion of an oral load of 500 ml of water (From A. C. Barger· *Metabolism, 5*:480, 1956.)

Barger has also demonstrated that in severe experimental right heart failure water diuresis is blunted. However, water excretion is by no means depressed to the same extent as sodium excretion. Total cumulative water excretion in these same experimental animals is shown in Figure 20B. Tricuspid insufficiency alone pro-

two diagrams to the right represent the two possible causes of glomerulo-tubular imbalance characterized as tubular preponderance. Reduced excretion, independent of the cause, leads to expansion of extracellular stores of salt and water and to the accumulation of edema.

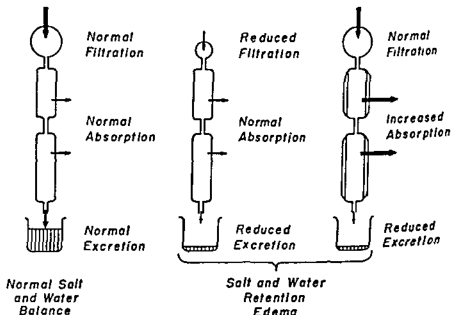


Fig. 19. The concept of glomerulo-tubular balance in relation to regulation of salt and water reserves of the body.

Limitation of Excretory Capacity for Salt and Water in Experimental Congestive Failure. Barger has recently shown that chronic congestive right heart failure may be induced in the dog by the combined lesions of a 50 per cent stenosis of the pulmonary artery and avulsion of the leaflets of the tricuspid valve. Such animals exhibit elevated right atrial pressure, reduced cardiac output, reduced exercise tolerance, ascites and edema.

The data shown in Figure 20A illustrate the progressive reduction in the capacity of an animal to excrete a standard sodium load when first, tricuspid insufficiency and later, pulmonary stenosis are induced. In control experiments, normal dogs excreted

Metabolic factor. Among the *Hormones*, those of the adrenal cortex, the antidiuretic hormone, and both epinephrine and norepinephrine have been implicated as playing causal roles.

Present evidence indicates that two factors, namely reduction in glomerular filtration rate and increased secretion of the adrenal salt retaining steroid, aldosterone, are the primary causes of the glomerulo-tubular imbalance which results in salt retention in edema. Renal anoxia, renal venous congestion, and stimulation of sodium reabsorption by renal nerves and/or by adrenal medullary amines play minor roles, if indeed they are of any significance in the pathogenesis of edema. In the interest of brevity and to avoid an overly contentious discussion, only the major factors will be considered in any detail. However, the role of antidiuretic hormone must be treated briefly.

Antidiuretic Hormone. The oft repeated observations that edematous patients exhibit delayed and depressed water diuresis and that plasma sodium and osmolality are frequently subnormal in severe congestive failure, in cirrhosis with ascites, and in nephrosis suggest excessive antidiuretic hormone activity. Some have gone so far as to state that water retention in edema is primary and that salt retention is secondary to body dilution. A more reasonable view is that excessive antidiuretic hormone activity may be a contributory cause of the hyponatremia of severely ill patients, but that sodium retention, not water retention is the basic abnormality in edema.

Goodman and Gilman were the first to demonstrate the presence of an antidiuretic substance in the urine and to show that its rate of excretion is related to the need for water conservation. Subsequently, excessive rates of urinary excretion of antidiuretic substances were noted in patients with cirrhosis and ascites by Ralli, Lloyd, and Sims, with congestive failure by Bercu and Dochios; with eclampsia by Teel and Ham, with hypertension by Grollman and with Bright's disease by Robinson. In general it has been obesrved that rate of excretion of antidiuretic substances is high during the phase of active accumulation of edema and low during the diuretic phase. In cirrhosis, antidiuresis has been variously ascribed to reduced destruction of hormone by damaged liver

duced no significant alteration in water diuresis. Excretion of water was normal, in fact slightly greater, in the dogs with the single lesion. On the other hand, the combined lesions of tricuspid insufficiency and pulmonary stenosis reduced the diuretic response to a standard water load by roughly one half. Again Barger's findings in experimental right heart failure are reminiscent of those of Schemm, Proger and others who have shown that patients in congestive failure excrete a water load somewhat less readily than do normal subjects.

FACTORS LIMITING SALT AND WATER TOLERANCE

In Table V are listed a number of factors to which this marked reduction in sodium and moderate reduction in water tolerance have been ascribed. These factors include the *Hemodynamic* ones of reduced renal blood flow, reduced glomerular filtration rate, and elevated venous pressure. Since changes in renal blood flow and filtration rate may well be mediated in part through the sympathetic nervous system, the second term, *Neural Factor* is used in a restricted sense to mean a direct control of the reabsorptive activities of the renal tubules by nerve impulses. Relative anoxia, as a consequence of renal ischemia, was long considered a dominant

TABLE V

RENAL FACTORS IN THE RETENTION OF SODIUM AND WATER IN EDEMA

I. *Hemodynamic* (neurally and hormonally mediated)
   1. Reduction in Renal Blood Flow and Glomerular Filtration Rate
   2. Elevation of Renal Venous Pressure

II. *Neural* (independent of hemodynamic)
   1. Nervous Stimulation of Renal Tubular Reabsorption of Sodium

III. *Metabolic*
   1. Anoxia

IV. *Hormonal*
   1. Aldosterone Excess
   2. Pitressin Excess
   3. Epinephrine and Nor-Epinephrine Excess

and osmo-regulatory mechanisms are not entirely independent. Peters maintains that although the hypothalamic-hypophyseal antidiuretic hormone mechanism is primarly responsive to changes in osmolality, it also responds to absolute or relative inadequacy of blood volume. The severely ill patient, or the patient subjected to massive paracentesis or diuresis may sacrifice normal osmolality to expand volume. Whether such a patient secretes excessive quantities of antidiuretic hormone in an absolute sense is uncertain. Relative to needs for maintenance of normal osmotic relationships, the secretion of any hormone is excessive.

*Reduction in Renal Blood Flow and Glomerular Filtration Rate.*That alterations in renal hemodynamics might play a significant role in the pathogenesis of edema in congestive failure was first clearly expressed more than a decade ago by Warren, Stead, Merrill, Mokotoff and others. They observed that renal blood flow is markedly reduced and glomerular filtration rate moderately reduced in edematous patients in severe congestive failure. Most of their patients with filtration rates less than 70 ml. per min. were frankly edematous. Those with greater filtration rates, especially those with rates approaching the normal range of 120 to 140 ml. per min. were not. They concluded that reduction in filtration rate without equivalent reduction in tubular reabsorptive activity leads to retention of salt and water and to the formation of edema.

Moderate exertion, in fact even the assumption of the erect posture, leads to an appreciable decline in renal blood flow and glomerular filtration rate in the normal subject. According to Merrill and Cargill, a number of patients who are compensated and edema-free when activity is restricted, suffer a marked fall in glomerular filtration rate to or below the presumed critical level of 70 ml. per min. and expand extracellular fluid reserves when activity is greater and more prolonged. Renal hemodynamic changes include constriction of both afferent and efferent glomerular arterioles. Constriction of afferent arterioles lowers filtration pressure and reduces filtration rate; constriction of both afferent and efferent arterioles reduces blood flow to a more marked degree than filtration rate. These hemodynamic changes have been ascribed on the one hand to enhanced vasoconstrictor nerve

tissue and to enhanced secretion of hormone due to the presence in serum of high concentrations of ferritin. In a few instances the concentration of antidiuretic substances in the serum of edematous patients has been observed to be greater than normal.

This seemingly convincing evidence has been called into question by Van Dyke who points out the lack of specificity of the assay methods employed. For the most part, it has not been proved that the antidiuretic materials are hormonal in nature and derived from the neurohypophysis. It is thoroughly possible that the materials extracted from the urine of edematous patients cause the liberation of hormone from the neurohypophysis of the assay animal and are not in themselves antidiuretic.

Recently Laragh has shown that ascites can be produced in the dog with diabetes insipidus by constriction of the inferior vena cava above the liver. These animals continue to show marked polydipsia and polyuria during accumulation of ascitic fluid, the rate of accumulation being proportional to salt intake, not water intake. Apparently the accumulation of ascites is in no wise dependent on the presence of an intact neurohypophysis, much less on the presence of an overactive one. A great deal of evidence points to the fact that alcohol produces its familiar diuretic response by inhibiting the release of antidiuretic hormone from the pituitary gland. Lamdin, however, has found that even repeated doses of alcohol to patients with congestive failure, cirrhosis, and nephrosis, do not restore the diuretic response to normal; in fact do not affect it at all. The implication is that excessive circulating antidiuretic hormone activity plays no role in the reduced diuretic response of edematous patients.

It is the authors personal view that antidiuretic hormone basically play the same role in the edematous patient that it plays in the normal individual. It is secreted in amounts sufficient to control water excretion and to maintain osmolality of the body fluids within normal limits. Its presence in excess is by no means necessary for accumulation of edema and ascites. Delayed and depressed diuresis may be a consequence of glomerulo-tubular imbalance rather than of excessive antidiuretic hormone activity. However, it was pointed out in Chapter V that volume regulatory

activity, on the other, to the liberation of renin by the kidney and the formation of the vasoconstrictor material, angiotonin, in the bloodstream.

Kattus, Briggs and others, observing recovery of compensation and loss of edema without increase in renal blood flow or glomerular filtration rate, have minimized the significance of renal hemodynamic factors in the pathogenesis of edema in congestive failure, or have even denied their existence. However, the fact that reduction of filtration rate does indeed lead to more complete reabsorption of sodium and water by the renal tubules has been amply demonstrated in experimental animals by Selkurt, Duggan, Mueller, Thompson and others.

In the studies of Thompson, a Dotter-Lukas balloon catheter was introduced into the femoral artery of a dog and positioned in the aorta immediately rostral to the origins of the renal arteries. By inflating the balloon, the renal arterial pressure could be reduced to, and stabilized at any desired value. Figure 21 summarizes the effect of controlled inflation of the balloon on renal arterial pressure, glomerular filtration rate, urine flow and sodium excretion in a respresentative experiment on an anesthetized dog. Prior to and during this experiment the animal was infused with isotonic saline at a rate of 10 ml. per minute. As a consequence of saline loading, high rates of urine flow and sodium excretion were observed in the initial two control periods. Reduction of renal arterial pressure from 150 to 90 mm. Hg. reduced filtration rate to a minor degree, namely from 77 to 70 ml. per min. However, this 10 per cent reduction in filtration rate was associated with a 50 per cent reduction in sodium excretion. Thus sodium excretion decreased from 900 to 450 μEq. per min. Inflation of the balloon a second time, reducing renal arterial pressure to 70 mm. Hg, caused a further decline in filtration rate and sodium excretion. Inflation a third time reduced filtration rate roughly by half, that is, to 40 ml. per min. Excretion of sodium to all intents ceased.

Thompson studied the renal response to a reduction in renal perfusion pressure in normal, in sympathectomized, in adrenalectomized, and in diabetes insipidus dogs. Under all experimental conditions, sodium excretion and urine flow decreased to essentially

the same extent in response to a reduction in filtration rate. Decreased excretion resulted from a relative increase in tubular reabsorption of salt and water. The response was obviously independent of renal nerves, the posterior lobe of the pituitary, and the adrenal glands; it appeared to be primarily related to the reduction

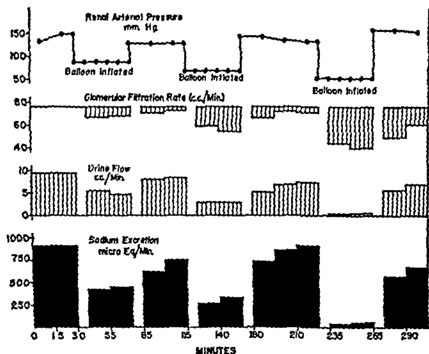


Fig. 21 The effects of controlled reduction of renal arterial pressure in the dog on glomerular filtration rate, urine flow and sodium excretion. (Drawn from data of D.D. Thompson and R.F. Pitts. *Am J Physiol.*, *168*:490, 1952.)

in filtration rate. At a filtration rate of 50 per cent of normal, roughly comparable to Merrill's value of 70 ml. per min. in man, reabsorption of sodium and water was essentially complete.

These experiments of Thompson demonstrate that an acute reduction in filtration rate, similar to that which occurs on assuming the erect posture and on mild to moderate exertion in the patient with reduced cardiac reserve leads to an abrupt reduction in the excretion of salt and water. What is the significance of

reduction in filtration rate in the long term regulation of fluid and electrolyte balance?

Mueller has shown that constriction of one renal artery in a dog with ureters separately exteriorized results in a reduction in glomerular filtration rate and in sodium excretion on the constricted side which persists for weeks. However, no change in either volume or composition of the body fluids occurs, for the normally functioning kidney maintains proper balance. When the normal kidney is removed, salt reabsorption is reduced and salt exretion increased on the side of renal artery constriction, presumably in response to a change in volume or composition of the extracellular fluid. Glomerulo-tubular balance is re-established by a reduction in tubular reabsorptive activity.

One may interpret the role of reduced filtration rate in the pathogenesis of edema in the light of these findings as follows. An acute reduction in filtration rate leads to salt and water retention and to a slight but significant expansion of extracellular fluid volume. The otherwise normal individual responds within a day or so by decreasing his rate of secretion of salt retaining adrenal steroids. Glomerulotubular balance is restored by reduction of tubular reabsorptive activity. No appreciable edema fluid accumulates. Certain patients with reduced cardiac reserve, under the stress of exertion, of infection or of progress of their disease, suffer an equivalent reduction of filtration rate. They do not reduce their rate of secretion of steroids, at least not to a degree sufficient to re-establish glomerulo-tubular balance. Accordingly, edema fluid accumulates.

Increased Secretion of Adrenal Cortical Steroids. Following the demonstration by Loeb and by Harrop of the significance of the adrenal cortex in the renal tubular reabsorption of sodium, there have appeared numerous references to the possible role of increased adrenal cortical activity in the pathogenesis of edema in congestive failure, cirrhosis, pre-eclampsia, nephrosis and nephritis. This view first gained strength from the observation that Addisonian patients, overtreated with desoxycorticosterone became edematous. More recently it has found support in the appearance of Cushing's syndrome in patients treated with large doses of

cortisone or ACTH. Several additional lines of evidence have implicated an adrenal cortical factor. In edematous patients, whatever the underlying disease, sweat and salivary sodium concentrations and fecal sodium excretion are low. Such effects can be induced in otherwise normal individuals by the administration of salt retaining steroids. According to Luetscher, Bartter and others, the urinary excretion of the salt retaining factor, aldosterone, is

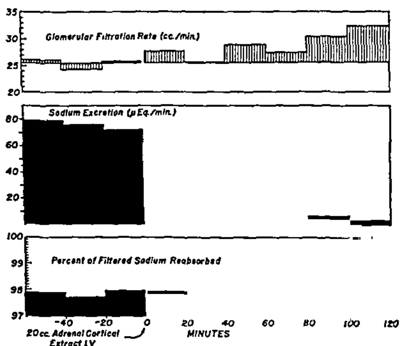


Fig 22 The effects of intravenous administration of whole adrenal cortical extract on glomerular filtration rate and reabsorption and excretion of sodium in the adrenalectomized dog (Drawn from data of J.C. Roemmelt, O.W. Sartorius, and R.F. Pitts *Am. J. Physiol.*, 159 124, 1949)

increased in congestive failure, cirrhosis, nephrosis and nephritis. Finally bilateral adrenalectomy may cause diuresis and loss of edema and ascitic fluid in dogs with experimental ascites, and in hypertensive and cirrhotic patients

One must remember that adrenal hormones affect the renal tubular reabsorption of a very small, though highly significant

fraction of the filtered sodium. The experiment on an adrenalectomized dog, summarized in Figure 22, was performed by Roemmelt some years past when cortisone was still unobtainable and aldosterone was an unknown component of the amorphous fraction. Replacement hormone therapy was withdrawn 4 days before the experiment. For 3 days the dog was allowed 0.6 per cent saline ad lib, the last day, only tap water. During the 3 control periods, 70 to 80 μEq. *of sodium were excreted per min.* This represented a failure to reabsorb only 2 per cent of the filtered sodium, for as shown at the bottom of the graph, reabsorption was 98 per cent complete. However, this minor defect in sodium reabsorption is highly significant; were it maintained over a period of days, extracellular reserves of salt and water would be seriously, possibly fatally depleted. At the break in the graph, 20 ml. of whole adrenal cortical extract was given intravenously. After a lag phase of 40 minutes, sodium reabsorption increased to become 99.9 per cent complete and urinary sodium excretion decreased essentially to zero.

Were such low rates of excretion of sodium to be maintained in the face of luxus intake, body sodium stores would of course progressively expand. To a certain extent this must occur in the Addisonian patient overtreated with desoxycorticosterone and in the patient with normal adrenal function treated with large doses of cortisone or ACTH. Why does it not occur in patients with primary aldosteronism, in some of whom higher rates of urinary excretion of salt retaining hormone are observed than in patients in congestive failure? Why cannot one make a normal dog or a normal man edematous with large doses of desoxycorticosterone?

The answer in part may be that the normal individual exposed to high salt retaining hormone activity increases filtration rate and filtered sodium *load sufficiently to offset increased tubular* reabsorption. The patient in congestive failure does not. We have thus come full circle in our argument. The patient in congestive failure with low filtration rate does not reduce salt retaining hormone output sufficiently to compensate for his reduced filtered load of sodium. Similarly the patient in congestive failure with excessively high salt retaining hormone output does not increase

the filtered sodium load sufficiently to compensate for increased reabsorptive activity. In either instance, glomerulo-tubular imbalance exists and tubular preponderance accounts for the progressively expanding sodium stores. It is not surprising, therefore, that certain patients are edematous despite the fact that their filtration rates are normal. Nor should it be surprising to find that others are edematous despite normal rates of excretion of salt retaining hormone. When considering glomerulo-tubular balance, normal is strictly a relative term.

In a study of Bartter summarized in Figure 23A, a normal subject was depleted of sodium by maintenance on a low salt intake and by oral cation exchange resin. Urinary excretion of aldosterone was high, roughly 50 micrograms per day,[17] and urinary and fecal excretion of sodium were negligible over the first 6 days of the study. On days 9 through 14, 2 liters of isotonic saline were given intravenously each day. Urinary aldosterone excretion decreased promptly and both urinary and fecal sodium excretion increased. Reinstitution of low sodium intake led to an abrupt increase in excretion of aldosterone and to a reduction in urinary and fecal sodium losses. The normal individual obviously responds promptly to variations in salt content of the diet by altering urinary output of aldosterone. The edematous patient responds in a qualitatively similar fashion but more sluggishly and the hormonal regulatory mechanism seems set at a higher level of activity.

In a similar study on a patient in congestive failure summarized in Figure 23B, gradual weight loss and decline of venous pressure resulted from a low sodium diet and oral resin therapy. Even though the patient was frankly edematous at the start of the period of observation, his rate of excretion of aldosterone was appreciable, amounting to 10 μgm. per day. Had this individual not been suffering from circulatory inadequacy, it is logical to assume that a

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[17] It is tacitly assumed that urinary excretion of aldosterone reflects rate of secretion of hormone by [illegible]
in the body fluids [illegible]
mented that it can [illegible]
salt intake excretes f[illegible]
he may excrete from 10 to 50 μgm. per day. In contrast the patient in congestive failure excretes from 10 to 60 μgm. per day.

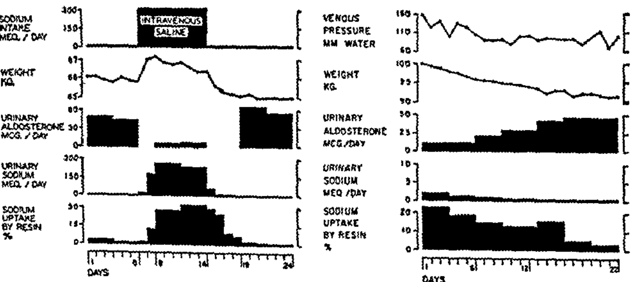


Fig 23. Effects of sodium deprivation and sodium loading on urinary and fecal excretion of sodium, on body weight, and on urinary excretion of aldosterone. A, Study on a normal man B, Study on a patient in congestive failure. (From L.E. Duncan, Jr., G.W. Liddle, and F.C. Bartter· *J Clin. Invest.*, 35.1299, 1956)

much less significant expansion of extracellular fluid volume would have caused his rate of excretion of hormone to drop essentially to zero. With progressive depletion of body sodium stores and an approach to a non-edematous state, urinary aldosterone excretion rose to high levels. Obviously the set of the mechanism controlling secretion of aldosterone is different in the patient with reduced cardiac reserve Is this mechanism sensitive to the total body sodium store, to sodium concentration, to extracellular volume or to some derivative of extracellular volume such as intravascular volume or pressure?

According to Bartter, Mach, Muller, Vesin and others, the mechanism regulating aldosterone secretion is sensitive to extracellular volume or to some derivative of volume. As shown in Table 6, urinary excretion of aldosterone is decreased by three procedures, each of which increases extracellular volume, namely the administration of water and pitressin, the administration of 3 per cent saline and the administration of isotonic saline. On the other hand, aldosterone excretion is increased by two procedures which reduce extracellular volume, namely water privation and mercurial diuresis. There seems to be no correlation of hormone excretion with intracellular volume, serum sodium and either intracellular or extracellular osmolality

One must temper ones enthusiasm for this thesis with the reservation that rate of urinary excretion may not be an infallable indication of rate of glandular secretion of aldosterone. Davis has shown in the dog, that less than 1 per cent of the glandular output is excreted in the urine. The possibility that any given factor may alter urinary excretion by affecting metabolism of hormone or renal tubular reabsorption of hormone rather than by affecting rate of glandular secretion must always be considered. Reduced hepatic destruction of hormone by the patient with cirrhosis or with an engorged congested liver is a likely but unproven possibility.

It is by no means accepted by all that the volume of extracellular fluid is the sole or even the major factor controlling aldosterone secretion. Deane, Singer *et al.*, and Laragh consider that the body store of potassium exerts a major controlling influence. a high

TABLE VI

REGULATION OF ALDOSTERONE SECRETION

	Extracellular Volume	*Intracellular Volume*	*Extracellular Osmolality*	*Intracellular Osmolality*	*Serum Sodium*
	Factors Decreasing the Secretion of Aldosterone				
Hydration plus pitressin	+	+	–	–	–
Infusion 3% NaCl	+	–	+	+	+
Infusion 0.9% NaCl	+	O	O	O	O
	Factors Increasing the Secretion of Aldosterone				
Thirsting	–	–	+	+	+
Mercurial diuresis	–	O	O	O	O

potassium store increasing, a low potassium store decreasing aldosterone secretion. Others have suggested that a receptor mechanism sensitive to serum sodium concentration regulates aldosterone output. The evidence for this view is not impressive. Perhaps as Farrell suggests, many factors can affect aldosterone secretion. Of these, the factor of volume of extracellular fluid is the one most pertinent to our discussion.

Role of Colloid Osmotic Force in Fluid Retention. Vander *et al* have recently postulated that retention of fluid and electrolyte in congestive heart failure is related to a greater reduction in renal blood flow than in glomerular filtration rate, i.e., to the increase in filtration fraction commonly observed in this condition. They point out that the filtration of a greater than normal fraction of the plasma perfusing the kidney results in an increase in the colloid osmotic pressure of the peritubular blood. They postulate that the oncotic force exerted by plasma proteins in peritubular capillaries is normally responsible for the reabsorption of fluid in the proximal tubule and that an increase in this force in congestive failure accounts for the over-reabsorption of fluid which results in edema.

To maintain proper perspective one must consider the quantitative aspects of their thesis. The oncotic force normally exerted by the plasma proteins in the peritubular capillaries is roughly 31 mm. Hg, slightly less than the equivalent of 2.0 mOsm. per liter concentration difference. Were filtration fraction to increase from the normal value of 0.2 to peak value of 0.5 in congestive failure, the colloid osmotic force would be increased to 50 mm. Hg, roughly the equivalent of 3.0 mOsm. per liter concentration difference.

Were any component of the tubular urine to be restricted in its movement relative to water to such an extent that its concentration increased by 2.0 mOsm. per liter in the normal or by 3.0 mOsm. per liter in the patient with congestive failure, all proximal transport would cease. One must remember that the total osmotic pressure of the proximal fluid is 5100 mm. Hg, equivalent to an osmolal concentration of 300 mOsm. per liter. It is difficult to believe that the tubular epithelium is so completely permeable to solutes as to permit passive migration of all in proportion to water

without restriction. Were the colloid osmotic force the only one available to cause reabsorption of fluid, excretory products could be concentrated to a negligible degree in the proximal segment.

Let us reverse the argument. As Bayliss has pointed out (see page 61), the osmotic force created by the active reabsorption of glucose from filtrate containing 100 mg. per cent is some 2.5 times the colloid osmotic force. In the mild or reasonably well controlled diabetic excreting no sugar but with blood glucose elevated to 200 mg. per cent, the osmotic force created by active reabsorption of sugar is some 5.0 times the colloid osmotic force. Were the tubule as freely permeable to salt and water as postulated by Vander *et al.*, mild diabetics would be considerably more edematous than patients in congestive failure.

In Chapter IV the author has developed the thesis that the reabsorption of sodium is active and provides the motive force for the reabsorption of the bulk of the filtered water in the proximal tubule. The active transport of any so-called threshold solute, such as glucose or amino acid, contributes to this force. Even the colloid osmotic force of plasma proteins in peritubular capillaries contributes, but the contribution is minor. It certainly cannot account for the reabsorption of four-fifths or more of the filtrate under normal conditions nor for fluid and electrolyte retention in congestive failure.

Compensatory Elements in the Fluid Retention of Edema. Why are renal salt retaining mechanisms so entrained in a variety of diseases as to lead to the formation of edema and ascites? Does expansion of extracellular fluid volume serve a useful purpose for the patient, is it an expression of the operation of a normal homeostatic mechanism carried to extremes in disease and therefore of limited use or actually deleterious for the patient, or is it strictly a pathologic result of disease serving no useful purpose whatever? No exact answer is possible. In our present state of knowledge, the question must be answered more on the basis of philosophy than of fact. However, Peters, Landis, Borst, and a number of others are more or less in agreement with the second of the possibilities outlined above; i.e., fluid retention is basically compensatory; carried to extremes of edema, it is deleterious.

As was pointed out in Chapter V, three simple procedures result in a prompt reduction in excretion of salt and water by normal subjects: quiet, erect standing, the application of venous tourniquets to the thighs, or the removal of a pint or so of blood from a peripheral vein. These same procedures induce a sensation of thirst. Common to all, are reduction in venous return, diminished distension of central and cephalic venous channels, and reduced cardiac output. Obviously, the circulatory status in each instance would be improved by increasing circulating blood volume. Presumably the volume receptor mechanism is triggered in some manner and through neural and humoral mechanisms, filtration rate is reduced, tubular reabsorption of salt and water is increased, and fluid intake is stimulated.

It is reasonable to assume that in diseases characterized by edema, circulating blood volume is less than optimum. This does not necessarily mean less than normal per Kg. of body weight. A patient with reduced cardiac reserve or one with cirrhosis, in whom blood is trapped in the portal system, might well have an increased total blood volume, yet an effective volume less than optimum. A patient with nephrosis or with protein undernutrition might well have a volume less than normal in an absolute sense due to failure of oncotic mechanisms to hold fluid in the vascular compartment The volume receptor mechanism, whatever and wherever it is, is triggered. Filtration rate decreases; adrenal cortical secretory mechanisms are activated, salt retention occurs. Thirst results and fluid intake increases. Slight hypo-osmolality develops, the more severe the disease process, the greater the demands for blood volume expansion and the more marked the hypo-osmolality. At best only one third of the retained salt serves a useful purpose in expanding blood volume, for plasma volume makes up a third or less of total extracellular fluid volume, two thirds or more of the salt and fluid is distributed in the tissue interstices as edema. The significant point is the following. Retention of salt and water in the edema of congestive failure and in the ascites of cirrhosis, no less than that which follows quiet standing, hemorrhage or the application of tourniquets to the legs may be basically compensatory insofar as it leads to expansion of blood volume. Insofar as it

abnormally expands interstitial fluid volume as edema, it serves no uesful purpose and is deleterious.

SUMMARY

At least two factors play dominant causal roles in retention of salt and water in edema. First, renal vasoconstriction, mediated by sympathetic nerve impulses or by renin release, causes reduction in renal blood flow and in rate of glamerular filtration. Reduction in filtered load, slowed transit and prolonged contact of the fluid with the tubular epithelium leads to over-reabsorption of salt and water. Second, an absolute or a relative increase in the secretion of salt retaining adrenal steroids stimulates tubular reabsorption and leads to retention of salt and water.

These two alterations in renal function are synergistic causes of the glomerulotubular imbalance which underlies the accumulation of edema. One cannot point to hypersecretion of aldosterone as the sole cause of fluid retention in a given patient, even though filtration rate is within normal limits. Were the individual otherwise normal, a compensatory increase in filtration rate would re-establish glomerulo-tubular balance despite enhanced tubular reabsorptive activity. Similarly one cannot point to a reduction of filtration rate, even though it falls to less than 50 per cent of normal as the sole cause of long term fluid retention in congestive failure. In the absence of circulatory insufficiency, the rate of secretion of salt retaining adrenal steroids would be reduced to a level sufficient to re-establish glomerulo-tubular balance.

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Part 2

Mechanisms of Action and Therapeutic Use of Diuretics

Chapter VII

INTRODUCTION TO DIURETIC THERAPY

IDEALLY, treatment of edema and ascites should be directed toward control of the primary disease and reversal of the pathophysiologic processes which cause expansion of extracellular fluid volume. Within limits, restriction of activity, reduction of weight, and limitation of salt intake can be considered as operating in this fashion in the decompensated cardiac, in some degree by improving the dynamics of the failing heart, in greater degree by reducing the demands upon it. The digitalis glycosides exert their major favorable effects by increasing the work capacity of the damaged myocardium. Steroid therapy in nephrosis and in the nephrotic stage of glomerulonephritis frequently induces remission of, and on occasions dramatically arrests the primary disease. Dietary management in cirrhosis may improve liver function, favorably alter nutrition, increase plasma protein concentration and induce remission of ascites. Diet, rest and salt restriction can in many instances prevent deterioration of the patient with mild pre-eclampsia.

The therapeutic goal of control of the primary disease can rarely be attained in the chronically or severely ill patient, and the physician must resort to diuretics to remove excess fluid from the body. A discussion of the overall management of the patient with edema and ascites is beyond the scope of this monograph; it is limited to a consideration of diuretic therapy and, more specifically, its physiological aspects.

Definition of Diuretic Agents. Diuretics are loosely defined as agents which increase the volume flow of urine. In this sense, water is the diuretic *par excellence*. However, diuretics are employed in therapeutics to eliminate excess body fluid and to reduce body weight. To achieve these ends by the administration of water, large quantities must be given; many substances are more

effective. A more precise mechanistic definition is that diuretics promote primarily the excretion of sodium and either chloride or bicarbonate, i.e., *those ions which are largely restricted to, and* which constitute the major electrolyte components of the extracellular fluid. Secondarily, water is eliminated in an amount equivalent to the ions excreted, reducing the volume of extracellular fluid and resulting in loss of weight. This definition properly emphasizes the facts that the excretion of ions is primary, that these ions are drawn from extracellular rather than cellular stores, and that increased urine volume and loss of weight are proportional to and the osmotic consequences of loss of ions.

Properties of the Ideal Diuretic. The ideal diuretic should have the following properties. (1) It should be potent, causing adequate diuresis and loss of weight in even the most severely ill patient, irrespective of the nature of his disease. (2) It should cause the excretion of sodium, potassium, chloride, and bicarbonate ions and water in the proportions in which they exist in extracellular fluid, it should cause no electrolyte imbalance due to the preferential excretion of one or another ion. (3) It should be active when used repeatedly; tolerance should not develop. (4) It should be active on oral administration. (5) A single dose should induce a relatively prompt diuresis. (6) It should be non-toxic even when given repeatedly over long periods of time. Needless to say, such a diuretic does not exist. However, there is reason to believe that compounds or combinations of compounds having these properties may eventually be found. Until they are, the practitioner must employ, as best he can, the agents at his disposal.

Classification of Diuretics. A functional classification of diuretics is presented in Table VII. This classification has the virtue that it illustrates the various means by which diuretic agents oppose [illegible] Diuretics, [illegible] ents which [illegible] as or more or less specifically antagonize enhanced tubular reabsorption of those ions. Either action tends to correct glomerulo-tubular imbalance. Filtered load can be increased either by increasing glomerular filtration rate or by increasing the plasma concentration of the filtered

ions. In part the action of colloids, digitalis glycosides and aminophylline is to increase glomerular filtration rate, hence to increase the filtered load of all ions. Ammonium chloride and calcium chloride, on the other hand, increase the plasma concentration of chloride at the expense of bicarbonate without increasing the plasma

TABLE VII

FUNCTIONAL CLASSIFICATION OF DIURETICS

A. *Physiological Diuretics*
   1. Diuretics which increase the filtered load of sodium and/or chloride ions.
      a. Colloids albumin, dextran, P.V.P., etc.
      b. Cardiac stimulants digitalis glycosides
      c Vasodilators of afferent glomerular arterioles aminophylline
      d. Acidifying agents· cation exchange resins, ammonium chloride
   2. Diuretics which more or less specifically antagonize over reabsorption of sodium and/or chloride ions
      a Aldosterone anti-secretory agents. amphenones
      b. Antialdosterone steroids SC5233, SC8109.
      c. Agents which increase the velocity of tubular flow in the most distal part of the nephron water.
      d. Agents which introduce a limiting ion gradient, osmotic diuretics mannitol, urea

B *Pharmacological Diuretics*
   1. Diuretics which specifically inhibit transport mechanisms for sodium and/or chloride ions
      a. Xanthines theophylline, theobromine, caffeine.
      b. Aminouracils aminoisometridine.
      c Mercurial diuretics· meralluride, mersalyl, etc.
      d. Chlorothiazide, hydrochlorothiazide.
   2 Diuretics which interfere with hydrogen for sodium exchange.
      a. Potassium salts KCl, KNO_3, etc.
      b. Carbonic anhydrase inhibitors acetazoleamide, chlorothiazide, dichlorphenamide.

concentration of sodium; hence these agents increase only the filtered load of chloride.

Antagonism of enhanced tubular reabsorption of sodium is most specifically accomplished by certain antialdosterone steroids which bind to renal tubular cells at those sites where aldosterone binds. These antisteroids displace aldosterone and reduce tubular reabsorption of sodium The aldosterone antisecretory agents, in con-

trast, depress the secretion of salt retaining hormone by the adrenal cortex. Water in large amounts impairs slightly the reabsorption of the last traces of sodium in distal tubules and collecting ducts, perhaps in part by increasing the velocity of flow and by diminishing the time of contact of the fluid with the tubular epithelium. Osmotic diuretics, such as urea, mannitol, hypertonic glucose, etc., limit the proximal tubular reabsortion of sodium and water and cause the delivery of excessive quantities of fluid and electrolytes into more distal parts of the nephron. Reabsorption distally is therefore incomplete. With the exception of the digitalis glycosides in congestive heart failure, the *Physiological Diuretics* exhibit a relatively low order of activity. At present they are most useful in supplementing other forms of therapy, although the antialdosterone steroids and antialdosterone secretory agents may well be harbingers of the diuretics of the future.

Diuretics, designated as *Pharmacological* in Table VII, include all of the potent inhibitors of tubular ion reabsorption. They correct glomerulo-tubular imbalance by reversibly inhibiting enzyme systems concerned with ion transport. They logically divide into two groups: one inhibiting sodium and chloride reabsorption, the other inhibiting sodium and bicarbonate reabsorption. There is reason to believe that at least three of the four classes of inhibitors of sodium and chloride transport have different mechanisms of action. In contrast, the sulfonamides all block the reabsorption of sodium and bicarbonate by virtue of their inhibition of carbonic anhydrase. Chlorothiazide has certain properties common to the *two major groups of agents, in that it may block reabsorption* of sodium and both chloride and bicarbonate ions.

Use of Diuretics. Diuretics are employed for the relief of *generalized edema and find their greatest use in such* chronic conditions as congestive heart failure, cirrhosis with ascites, and the nephrotic syndrome, including the nephrotic stage of nephritis and so-called genuine lipoid nephrosis. They are also useful in certain acute, self limited conditions sucn as toxemia of pregnancy, and in periodic recurrent edema, e.g., premenstrual edema. They are less frequently employed for dehydration in epilepsy and to reduce regional edema, e.g., that accompanying thrombophlebitis.

Diuretics other than water are of no value in reducing the nitrogen retention of chronic renal insufficiency, for they do not, in the usual sense, increase renal excretory functions other than that concerned with salt elimination. Under no circumstances should any diuretic be administered to an anuric or markedly oliguric patient, for none can initiate the flow of urine, and all are, in variable degree, toxic when retained in the body.

Salt Restriction in Diuretic Therapy. Since diuretics are employed primarily to establish a negative salt balance by promoting the excretion of sodium, the overall efficacy of therapy can be considerably enhanced by restricting the dietary intake of this ion. In fact, whenever diuretics are used, salt intake should be reduced at least to some extent. If the degree of salt privation is adequate, fluid restriction is unnecessary, inadvisable, and in fact, inhumane. An exception to this rule is the treatment of bromidism, where high salt and water intake, combined with mercurial diuresis, is employed to rid the body of bromide. In practice, it is often convenient to permit a moderate intake of salt in order to provide a more palatable diet and then to remove that salt from the body by diuretic therapy.

Chapter VIII

COLLOIDS AS DIURETICS

EDEMA and/or ascites are commonly associated with and often roughly proportional to hypoproteinemia in cirrhosis, in the nephrotic state, and in protein malnutrition. In these conditions excessive transudation of fluid into the peritoneum and tissue interstices has in the past been explained solely or largely in terms of an imbalance of forces across the capillary endothelium created by low colloid osmotic (oncotic) pressure of the blood plasma Some have claimed that edema or ascites will accumulate if the plasma concentration of albumin is below 3 gm. per cent and that fluid will be absorbed into the vascular compartment if concentration is above this critical level. In cirrhosis, increased portal and intrahepatic capillary pressures constitute additional factors directing fluid into the peritoneum. Without in any sense detracting from the significance of hydrostatic and oncotic forces in determining transudation of fluid, it has become increasingly apparent that retention of salt and water in the edema and ascites of nephrosis and cirrhosis is no less dependent on altered renal function than it is in congestive heart failure (see Chapters III, V, and XII). Spontaneous diuresis has been observed to occur at a time when the concentration of plasma albumin is below 3 gm. per cent No diuresis may occur when plasma albumin is restored to a range significantly above the so-called critical level. It is not surprising, therefore, that the treatment of patients with nephrosis and cirrhosis with salt poor concentrated human albumin has not been uniformly successful in relieving edema and ascites. Hypoproteinemia is a contributory factor in edema and ascites; it is not a complete explanation of fluid retention in itself.

Use of Albumin in Patients with Cirrhosis and Ascites has, with few exceptions, been unrewarding. Janeway observed no loss

of ascites in 6 patients with cirrhosis who received intravenously from 350 to 950 gm. of salt poor concentrated human serum albumin in daily doses of 50 gm. Thorn, Gibson, and Kunkel noted that occasional patients, after receiving numerous albumin infusions, exhibited diuresis and loss of ascites. Frequently patients with both edema and ascites lose interstitial but not peritoneal fluid in response to therapy. Patek observed that the concentration of protein in ascitic fluid increased in direct proportion to the increase in concentration in plasma during the intravenous infusion of albumin, an observation which is reasonable considering the relatively high permeability of liver capillaries to colloids. He noted that as much as 50 per cent of the administered albumin was transferred to the ascitic fluid. Although the oncotic effect of the proteins of plasma was restored to normal, ascites continued to collect at the usual rate. The intravenous administration of albumin increases plasma volume and no doubt elevates portal and intrahepatic capillary hydrostatic pressures as well. The combination of increased capillary pressure and increased colloid content of ascitic fluid offsets the beneficial effects of increased plasma protein. The administration of albumin and other colloids in cirrhosis with ascites is accordingly of little or no value.

Use of Albumin in Patients with Nephrosis has been somewhat more successful Janeway, Thorn, Luetscher, Riley, Siegal, Chinard, Eder, Lauson and their respective colleagues have observed diuresis and loss of edema in 50 to 70 per cent of selected patients receiving daily infusions of 25 to 75 gm. of salt poor human serum albumin. The albumin has usually been administered in 10 per cent solution in isotonic glucose over a period of 2 hr. or so. In the responsive patient, water diuresis follows each albumin injection. At first relatively little sodium and chloride are excreted. With repeated daily infusions, successive water diureses are accompanied by greater and greater salureses. It is interesting that the peak of each water diuresis precedes that of its accompanying saluresis 2 hours or more. With loss of salt, more profound water diuresis occurs, weight is lost and edema disappears. The most favorable responses occur in patients who exhibit no azotemia, and who have

normal or supernormal rates of glomerular filtration prior to therapy.

All who have measured plasma volume have found it increased following albumin infusion. However, Chinard has pointed out that the degree of expansion of volume is less than the theoretic iso-oncotic value. He suggests that, in consequence of the expanded plasma volume, capillary hydrostatic pressure rises. The outwardly directed filtering force increases, in part balancing the inwardly directed oncotic force and resulting in less than theoretic expansion of volume. Because of the very great permeability of the glomerular capillary membranes to protein in nephrosis, much of the albumin administered by vein is lost during the 24 hr. following the infusion. High cost of salt poor albumin and excessive urinary wasteage make this form of therapy impractical except on an experimental basis.

The water diuresis which almost immediately follows injection of albumin is no doubt related to expansion of plasma volume. Although the titre of ADH in plasma is primarily responsive to the osmolality of the body fluids, it is also affected by plasma volume or some derivative of volume (see Chapter V). According to Henry and Gouer, distension of the left atrium inhibits ADH release reflexly and causes water diuresis. Such atrial distension might well result from the injection of hyperoncotic albumin.

Eder and others have observed a high degree of correlation between increased rate of glomerular filtration and diuresis of water and salt in both spontaneous diureses and in those associated with the repeated infusions of albumin. These findings suggest that the glomerulo-tubular imbalance which underlies water and salt retention in nephrosis is due in some patients to a relative deficiency of glomerular filtration.

That enhanced tubular reabsorption plays some role in salt and water retention is equally evident. Burnett et al and Metcoff et al have shown that when PAH or thiosulfate are administered to nephrotic patients as sodium salts, they are largely excreted in combination with potassium. Normal subjects, in contrast, excrete them in the form in which they are given. Ingbar has shown that the administration of ACTH and cortisone causes the excretory

pattern of the normal subject to approximate that of the nephrotic. These facts, coupled with the observation of Luetscher and others that large amounts of aldosterone are excreted in the urine of patients with nephrosis, suggest that the glomerulo-tubular imbalance is in part due to enhanced exchange of sodium for potassium and no doubt for hydrogen and ammonia as well. However, in even greater degree, it is due to over-reabsorption of sodium and chloride as ion pairs. The infusion of albumin is associated with a reduction in urinary excretion of aldosterone, with increased sodium output, and with a decrease in body weight. The relative importance of increased filtration and of decreased tubular reabsorption of salt and water as causes of diuresis cannot be assessed at the present time. No doubt both are significant.

Use of Dextran and Other Colloids in Nephrosis. As was mentioned above, high cost and inadequate supply render therapy with salt poor human serum albumin impractical except on an experimental basis. Three colloids have been successfully substituted in the treatment of nephrosis: polyvinal pyrrolidone, gelatin, and dextran. In the past, acacia (gum arabic) has been used. The latter is mentioned here only to condemn it most strenuously, for as Hueper, Mannix and others have shown, its use leads to a storage disease known as arabinosis, characterized by splenomegally, hepatomegally, depressed formation of plasma proteins and other liver dysfunctions. Of the three recommended compounds, gelatin and dextran are most favored in this country. Polyvinyl pyrrolidone is retained in the body for long periods of time, and seems on this score to be less desirable. All of these colloids are essentially similar to albumin in their actions. When given to patients with nephrosis, they produce a transient expansion of plasma volume, water diuresis followed by salt diuresis, increase in glomerular filtration rate, and no doubt decrease in aldosterone excretion as well. These compounds are commonly given intravenously in 10 to 12 per cent solution in isotonic glucose. James et al recommend a dose of 1.2 to 1.8 gm. of dextran per Kg. body weight, infused at a rate of 2 to 4ml. per min. These authors suggest that blood pressure be measured every 20 min. and if systolic

normal or supernormal rates of glomerular filtration prior to therapy.

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pressure increases to values in excess of 140 mm. Hg, that the infusion be stopped.

Albumin, dextran and other colloids merely promote the elimination of fluid in nephrosis; they do not alter the course of the disease. Remission of edema is short lived and courses of therapy must be repeated at frequent intervals.

Complications of Colloid Therapy. All colloids may induce severe hypertension and convulsions or pulmonary edema when given rapidly and in large amounts to patients with anasarca, due no doubt to the sudden attraction of large volumes of fluid into the vascular system. It is therefore advisable to administer them in smaller than usual amounts in the presence of massive edema and pre-existing hypertension. Dextran prolongs bleeding time, due to inhibition of prothombin activation, and may be associated with epistaxis and/or subcutaneous ecchymosis. Certain patients are sensitive to dextran and may exhibit allergic manifestation following the first infusion. Others may develop minor or major sensitivity during repeated courses of therapy.

SUMMARY

The intravenous administration of concentrated salt poor human serum albumin, of dextran or of gelatin to patients with nephrosis frequently induces water diuresis and subsequently saluresis and loss of weight. Repeated daily infusions are necessary to cause a significant response, for all colloids are rapidly eliminated in the urine in consequence of excessively high permeability of the glomerular membranes Plasma volume is expanded, glomerular filtration rate and renal blood flow are increased, and urinary excretion of aldosterone is reduced. Diuresis and loss of weight are results of both increased filtration and reduced tubular reabsorption of salt and water. Colloid therapy of cirrhosis with ascites is commonly ineffective due to rapid transfer of the oncotic agent into ascitic fluid. Since portal and intrahepatic capillary hydrostatic pressures increase with increased plasma volume, and since the colloid is transferred to ascitic fluid, no significant reabsorption of fluid from the peritoneum occurs, even though the colloid osmotic pressure of the plasma is increased to normal levels.

crease. However, certain patients exhibit significant diuresis without appreciable changes in these discrete renal functions. One such study on a patient in congestive failure is summarized in Figure 24. The administration of equivalent doses of digoxin to normal subjects and to patients with cirrhotic and nephrotic edema produced a perceptible but less significant diuresis of salt and water with no change in renal blood flow or filtration rate. Farber's observations

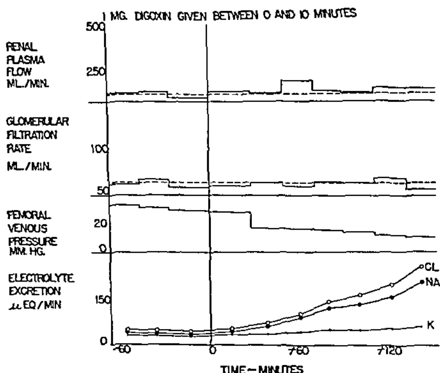


Fig. 24. The diuretic effects of the intravenous administration of 1.0 mg of Digoxin in a patient in congestive failure. Increased excretion of sodium and chloride in the absence of change in renal plasma flow and glomerular filtration rate suggests a direct effect of the glycoside on the kidneys. (From S J. Farber, J.D Alexander, E.D. Pellegrino, and D.P Earle *Circulation*, 4.378, 1951)

strongly imply that at a constant filtered sodium load, reabsorption is slightly depressed and excretion slightly increased by a direct action of digoxin on the renal tubules. The effect is more marked in patients in congestive failure than in those with nephrotic or

Chapter IX

DIGITALIS GLYCOSIDES

A NUMBER of plant and animal extracts containing cardiac glycosides have been employed as folk remedies over the centuries. Squill was known to the ancient Egyptians and was used by the Romans as a diuretic, heart tonic, emetic and rat poison. Dried toad skin, containing the glycoside bufagin, was both a Chinese and Western folk medicine. Digitalis or foxglove, while long used by the Welsh, became popular as a treatment of dropsy following the classic description of its actions by William Withering in 1785. Withering was aware that digitalis was not equally effective in all forms of dropsy, but apparently did not recognize that his successes were restricted to patients with heart disease. John Ferriar in 1799 was the first to ascribe to digitalis a primary cardiac action and to relegate to a secondary position its diuretic effects.

Today the cardiac glycosides are not generally regarded as diuretics in the true sense of the word. The author has chosen to classify them as physiological diuretics, implying that they restore the work capacity of the heart and counteract the circulatory deficiencies which lead to glomerulo-tubular imbalance and to compensatory retention of salt and water. It is widely accepted that the cardiac glycosides have no place in the therapy of edema other than that associated with congestive circulatory failure. The actions of these drugs on the heart and circulation which secondarily result in diuresis and discharge of edema fluid are outside the scope of this monograph.

A Direct Diuretic Action of Cardiac Glycosides has recently been described. Farber and his associates noted that the intravenous administration of 1.0 to 1.5 mg. of digoxin to patients in congestive failure causes a prompt diuresis of sodium and water. Frequently renal blood flow and rate of glomerular filtration in-

action could be ascribed to suppression of aldosterone stimulation of tubular reabsorption.

However, another interpretation is possible. Schatzmann first showed in 1953 that strophanthin prevents the uptake of potassium and the elimination of sodium that normally occurs when cold stored red cells are incubated with glucose at 37°C. The drug does not affect oxygen consumption nor lactic acid production, hence inhibits ion transport beyond the stage of energy production. Joyce and Weatherall and Kahn and Acheson showed that other cardiac glycosides including digoxin exert the same effect. Glynn has presented more direct evidence that the cardiac glycosides interfere with the ion pump rather than its energy source. Neither desoxycorticosterone nor aldosterone modify the action of digoxin on red cells.

It is impossible at the moment to decide which thesis applies to the renal tubule, or in fact whether either does. Furthermore, it is impossible to assess the relative significance of the direct renal action and of the cardiac stimulating action of the digitalis glycosides in explaining their diuretic effects in [illegible]
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on the heart The unsaturated lactone *portion* is necessary and either splitting the ring or saturating it destroys activity. The steroid nucleus bears a superficial resemblance to desoxycorticosterone and aldosterone. One might speculate that digoxin binds to the same receptor sites of renal tubules which bind aldosterone, displacing the salt retaining steroid from its combination Digoxin could then be classified as an antialdosterone and its direct diuretic

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It is impossible at the moment to decide which thesis applies to the renal tubule, or in fact whether either does. Furthermore, it is impossible to assess the relative significance of the direct renal action and of the cardiac stimulating action of the digitalis glycosides in explaining their diuretic effects in patients wth congestive circulatory failure.

SUMMARY

The cardiac glycosides have a clinically significant diuretic action only in congestive heart failure and presumably exert their major effects through the improvement in cardio-circulatory dynamics which they induce. However, these drugs apparently depress renal tubular reabsorption of sodium chloride in modest degree. It is possible that this action is antialdosterone in nature or *that the drugs block ion pumps of renal tubular cells more directly.* These findings should not be interpreted as justification for use of presently available glycosides in edemas other than those resulting from congestive heart failure.

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cirrhotic edema or in normal non-edematous controls. If filtration rate increases in patients in congestive failure, the diuresis may be much more profound.

Hyman and his associates have recently demonstrated that the injection of 0.065 to 0.250 mg. of digoxin into one renal artery of an anesthetized dog produces diuresis of sodium and water which is restricted to the side of drug administration and which lasts for the 2 hr. period of observation. A greater response was obtained in one dog in congestive failure due to experimental mitral stenosis. Barger, in an as yet unpublished study, has extended these observations on unanesthetized dogs in congestive failure with ureters separately explanted in the abdominal wall and with a catheter fixed in one renal artery. Injection of 0.5 mg. of digoxin into one renal artery results in diuresis of sodium and water, most apparent on the side injected and lasting for more than 24 hr. Shatzmann, Windhager and Solomon have demonstrated that perfusion of the proximal tubule of Necturus with solutions containing minute amounts of ouabain inhibits the active transport of sodium. These several investigations are all consonant with the view that cardiac glycosides can inhibit tubular transport of sodium by a direct action on the kidney.

The Mechanism of Diuretic Action of Digoxin in the studies of Farber, Hyman, Barger, and Schatzman is by no means clear, but one may speculate along the following lines. Cardiac glycosides are complex molecules consisting of three basic components: a sugar moiety or glycone, a 5 or 6 membered unsaturated lactone ring, and a cyclopentanophenanthrene nucleus or steroid moiety. While it influences solubility and duration of action, the glycone is not necessary for the molecule to exhibit its characteristic effects on the heart. The unsaturated lactone portion is necessary and either splitting the ring or saturating it destroys activity. The steroid nucleus bears a superficial resemblance to desoxycorticosterone and aldosterone. One might speculate that digoxin binds to the same receptor sites of renal tubules which bind aldosterone, displacing the salt retaining steroid from its combination. Digoxin could then be classified as an antialdosterone and its direct diuretic

Chapter X

ACIDIFYING AGENTS

CATION EXCHANGE RESINS

IN the introduction to this section, it was pointed out that diuretics are administered to induce a negative balance of sodium, and that some restriction of sodium intake is imperative, if one is to attain this end. While preparation of a nutritious and palatable diet containing 2 to 3 gm. of salt per day is perfectly feasible, limitation of intake to 1.0 gm or less per day is virtually incompatible with a diet of adequate nutritive content and taste. Dock in 1946 first suggested that ion exchange resins, long used in industry for purposes of de-salination, might be administered to patients to prevent intestinal absorption and to promote fecal excretion of sodium. Not only might such resins permit a more liberal intake of sodium, they might also be useful to abstract sodium from the body by an enteric rather than by a renal route. The first of these ends is more frequently achieved in practice than is the second.

Chemical Nature of Ion Exchange Resins. An ion exchange resin is a crosslinked polymer which contains either acidic or basic groups and which, therefore, can exchange either cations or anions with the surrounding fluid medium. The resin has essentially infinite molecular size and its monomeric components are extensively crosslinked with stable carbon bridges. Accordingly, it is highly insoluble. The resins, useful clinically for removal of sodium from the gut, contain sulfonic, carboxylic or phenol acid radicals. They may be classified as phenol-formaldehyde, phenol-methylene, or polystyrene polymers. Those which exchange anions are polyamines and are sometimes added in small amounts to the cation exchange resins to reduce their acidic properties. The gen-

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3. Hyman, A. L., Jacques, W. E., and Hyman, E. S.: Observations on the direct effect of digoxin on renal excretion of sodium and water. *Am. Heart J., 52*:592, 1956.
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5. Schatzmann, H. J., Windhager, E. E., and Solomon, A. K.: Studies in single proximal tubules of the Necturus kidney. *II.* Effect of 2, 4-dinitrophenol and ouabain on water reabsorption. *Am. J. Physiol.*, to be published.

the ammonium chloride or potassium chloride formed is absorbed in the lower part of the digestive tract. In the less acid environment of the intestine, the hydrogen cycle resin is converted to sodium, potassium, calcium and magnesium cycles and excreted as such in the feces.

Theoretically, at the pH and concentration of the lower intestine, some 6 to 7 mEq. of cations should be bound to each gm. of resin Actually much less is bound; only 0.9 to 1.2 mEq. of sodium, ± 1.0 mEq. of potassium, and considerably smaller but by no means negligible amounts of calcium and magnesium are bound per gm. of resin. This failure to bind theoretical quantities is in part a result of very slow attainment of equilibrium due to the compact structure of the resin lattice. It is also probable that protein, amino acids, etc., inhibit ion uptake. As will be explained below, the colonic mucosa specifically abstracts sodium from the resin, reducing the quantity bound.

Binding of Ions in the Intestinal Lumen. From the work of Visscher and his associates on the dog, it is evident that considerable quantities of sodium exchange in both directions across the intestinal epithelium between blood stream and luminal contents during the transit of food and fluid along the intestine. Exchange proceeds at such a rate that the sodium present in the blood turns over or exchanges with that in the gut once every 90 min. This means that in a 70 Kg. man, 190 gm. of sodium is delivered into and abstracted from the gut each day. If one introduces into this stream of sodium 50 to 100 gm. of resin, it is remarkable that fecal excretion is not highly significant and that sodium is not rapidly abstracted from the body, especially in edematous patients maintained on low salt diets.

Visscher has demonstrated that the rate of exchange of sodium across the duodenal epithelium is high, and that both rate of transfer into the gut and out of the gut diminish through jejunum, ileum and colon. However, the ratio of rate of transfer into blood/rate of transfer into lumen increases progressively from oral to aboral ends of the intestine. The greater this transfer ratio, the more completely is sodium cleared from the lumen of the gut. The colon is a highly efficient absorber of sodium and the feces

eral structural pattern of a carboxylic cation exchange resin is illustrated in Figure 25.

Resins, like all other dissociated compounds, must obey laws of ionic equivalence. Figure 25 shows the several anionic sites of the resin neutralized with Na^+, H^+, NH^+_4 and K^+. These anionic

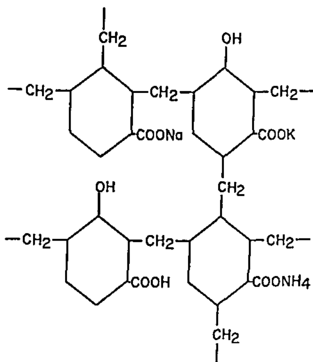


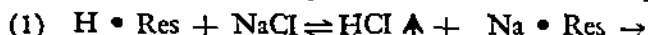
Fig. 25. Structure of a carboxylic cation exchange resin.

sites have differing affinities for the several ions found in the gut contents. Were all present in the same concentrations, the quantities of ions bound would be in the order $Ca^{++} > Mg^{++} > K^+ > Na^+$. However, the concentration of sodium in the gut considerably exceeds that of any other ion. Hence when the so-called hydrogen cycle resin (all anionic sites neutralized with H^+ ions) is administered, sodium is bound in greatest quantity. The binding of ions by carboxylic resins is especially dependent on pH. In the highly acid environment of the stomach, ammonium or potassium cycle resins are converted to the hydrogen cycle, and

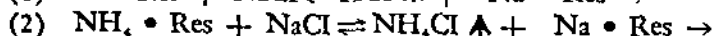
ably and excrete more sodium in the feces than is contained in the diet; they lose weight. Presumably their colonic mechanisms are less intensely stimulated to conserve salt.

Potassium is contained in digestive secretions in much higher concentration than in extracellular fluid, i.e., as much as 15 to 20 mEq per liter. When a resin is administered in its hydrogen cycle, it binds nearly as much potassium as sodium If sodium is excessively conserved due to adrenal stimulation, considerably more potassium than sodium may be eliminated. This results from the high affinity of resins for potassium, the relatively high concentration of potassium in digestive secretions, and perhaps, under adrenal stimulation, to an active exchange of potassium for sodium by the colonic mucosa. Significant quantities of calcium and magnesium are also bound by resin. The possibility exists that resins may also remove trace metals, riboflavin, and thiamine.

Alterations in Composition of the Body Fluids Induced by Resins derive directly from the ion binding properties described above. Hydrogen cycle and ammonium cycle resins induce hyperchloremic metabolic acidosis. The plasma bicarbonate concentration decreases from its normal level of 26 to 28 mEq. per liter to between 10 and 20 mEq. per liter The plasma chloride concentration increases from its normal level of 105 to as high as 115 to 120 mEq. per liter. Ordinarily, plasma sodium concentration is unchanged or is only slightly depressed. Plasma pH decreases moderately. These manifestations of acidosis produced by the ingestion of 45 gm. of hydrogen or ammonium cycles resin per day are comparable to those produced by 7 to 10 gm. of ammonium chloride per day. In fact they are the exact chemical equivalent.



(1) $H \bullet Res + NaCl \rightleftharpoons HCl \uparrow + Na \bullet Res \rightarrow$



(2) $NH_4 \bullet Res + NaCl \rightleftharpoons NH_4Cl \uparrow + Na \bullet Res \rightarrow$

As shown in equation (1), hydrogen cycle resin ($H^{\bullet}Res$) reacts with NaCl in the gut to form HCl, which is absorbed and neutralized in the body by intracellular and extracellular buffers, including bicarbonate. As shown in equation (2), ammonium cycle resin ($NH_4^{\bullet}Res$) reacts with NaCl to from NH_4Cl. This salt is absorbed, converted into urea and HCl in the liver, and the acid so formed is neutralized by body buffers. While the patient with

of man normally contain negligible amounts of this ion. It is probable that resin binds sodium in the oral end of the intestine and that sodium is removed and replaced with potassium and, to a slight extent, hydrogen in the aboral end.

The activity of the colon in removing sodium from feces and from resin is regulated by salt retaining adrenal cortical steroids, probably by aldosterone. Berger and his associates have shown that the rat, an animal which normally excretes fair amounts of sodium in the feces, completely absorbs this ion when treated with desoxycorticosterone. Normal human subjects, excreting significant amounts of sodium bound to resin, reduce fecal excretion essentially to zero when treated with DOCA. Emerson et al have shown that adrenalectomized patients or patients with Addisons disease who lack aldosterone excrete far more sodium in the feces when given resin than do normal subjects.

It has been general clinical experience that patients vary widely in their response to resins. Some excrete small amounts of sodium bound to resin, even less than the gm. or so contained in a very low sodium diet. Others may excrete greater amounts of sodium than are present in the food ingested. However, they rarely develop a significant negative balance. These findings have been loosely explained by the statement that the resin reacts differently with exogenous and endogenous sodium, i.e., that resins will remove a limited amount of dietary sodium in the feces, but that they will not abstract sodium from body reserves. In view of the rapid turn-over of sodium across the gut wall, this explanation is patent nonsense, if it is interpreted literally. A much more reasonable explanation is that the efficacy of resins in removing sodium from the body is inversely related to the intensity of stimulation of salt retaining mechanisms. If the adrenal salt retaining system is maximally activated, the concentration of sodium in sweat, saliva, urine, and colonic contents will be minimal. Essentially all of the sodium bound by resin in the upper gut will be abstracted in the colon. Restriction of dietary sodium intake stimulates sodium conservation by both normal subjects and by edematous patients. Liberalization of dietary intake reduces conservation and permits fecal loss of sodium on resin. Some patients respond more favor-

consideration of ammonium chloride therapy. (2) Compensation for diversion of ingested ions from a urinary to a fecal route of elimination Commonly on institution of resin therapy, urinary sodium excretion decreases. If the decrease in renal excretion is equal to the increase in fecal excretion, no net loss of body sodium occurs. Occasionally, however, urinary output of sodium is less markedly depressed; the sum of urinary and fecal excretions exceeds intake, and modest loss of weight occurs. Less frequently a significant diuresis of chloride, sodium and water results from the acidosis, and edema clears. In contrast, urinary potassium excretion is rarely reduced to a degree sufficient to balance fecal loses when ammonium or hydrogen cycle resins are given; hence body stores of potassium may be rapidly depleted.

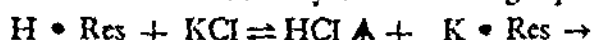
Toxicity. Cation exchange resins are by no means benign therapeutic agents. The sulfonic resins cause epigastric burning and discomfort due to their relatively strong acid properties and have now been replaced by carboxylic resins. These latter, though less irritating, may produce a sense of fullness, and rarely nausea and vomiting. The most common complaint is constipation, a difficulty which may be alleviated by administration of methylcellulose or other hydrophilic bulking agents. However, abdominal distension and constipation are frequently signs of potassium depletion and should be considered as such till proven otherwise. Fecal impaction is a possible hazard, especially in the elderly.

The mild to moderate acidosis which accompanies resin therapy is of little significance if the patient has normal renal function. However, increasing dyspnea, azotemia, and oliguria may be signs of severe acidosis in patients with inadequate renal reserve Weakness, lassitude, abdominal distension, constipation, tachycardia, and cardiac irregularities are signs of significant potassium depletion and may occur, although rarely, when the patient is receiving a part of the resin dose in the potassium cycle.

In acute renal failure potassium cycle resins are absolutely interdicted, although hydrogen cycle or sodium cycle resins are occasionally used to combat hyperpotassemia. The hydrogen cycle resins increase acidosis and are to be avoided if the patient is severely acidotic, the sodium cycle resins increase extracellular ion

normal renal function can and does compensate this acidosis and keep it within reasonable bounds, the patient with severely reduced renal function cannot. Such individuals may become extremely acidotic, exhibit Kussmaul breathing and become oliguric, azotemic, and comatose on prolonged resin therapy.

A somewhat more subtle disturbance produced by hydrogen and ammonium cycle resins is hypokalemia and depletion of tissue stores of potassium as indicated by the following equation.



$$H \cdot Res + KCl \rightleftharpoons HCl \uparrow + K \cdot Res \rightarrow$$

The degree of reduction of plasma concentration of potassium is unfortunately not an accurate indication of the extent of depletion of body potassium reserves, for large amounts of this ion may be removed from tissues with relatively minor alterations in plasma level Signs and symptoms referable to potassium depletion are discussed in Chapter XIX. All resins now marketed for removal of sodium in the gut contain roughly one-third of total resin in the potassium cycle. In the absence of vomiting, diarrhea or anorexia, this quantity of potassium is sufficient to protect the patient from excessive potassium loss. Administration of resin entirely in the potassium cycle would cause no change in acid base balance. One might, therefore, presume this to be the ideal form in which to administer resins. However, the incorporation of even one-third of total resin in the potassium cycle reduces overall efficacy of binding of sodium. Unfortunately, no alternative exists but to supply a part of the resin in the potassium cycle or to give potassium supplements; the two are equivalent.

Hypocalcemia has been no problem except in patients maintained on daily doses of resin for 6 months or more. Oral calcium supplements are of little value, for this ion is strongly bound to resin. Calcium in the form of the gluconate can be administered parenterally or resin can be given in interrupted courses with the view that calcium deficits can be made up during the drug-free interval.

Renal Responses to Resin Therapy fall into two general categories: (1) Compensations for hyperchloremic metabolic acidosis, namely low urine pH and high rate of excretion of titratable acid and ammonia. These factors will be discussed in Chapter XI in a

therapy, rendering more effective subsequent treatment with mercurial compounds. It can scarcely be considered a prime reason for the administration of resins; the same effect can be obtained at considerably less expense by administering ammonium chloride. According to this view, resins should be considered as adjuvants in the treatment of edema, not as primary therapeutic agents.

Others have had greater success with resins in the primary removal of edema fluid. They have noted in favorable cases that fecal elimination of sodium may exceed dietary intake and that diuresis of sodium, chloride and water may lead to rapid reduction of edema. Still others complain that relatively little liberalization of dietary sodium is possible without gain of weight and that resins are relatively ineffective in the primary removal of edema fluid. Resins have been employed with success and with failure in congestive heart failure, cirrhosis with ascites, nephrosis and the nephrotic stage of glomerulonephritis, pre-eclampsia and a variety of other diseases. The author would reconcile these conflicting views in the following terms. The adequacy of response to resin therapy is less determined by the nature of the primary disease process than by its severity as expressed in the intensity of stimulation of salt conserving mechanisms. The more intense the stimulation of salt conservation, the more avid the absorption of sodium by the colon, the more complete the reabsorption of sodium in the renal tubule and the less the fecal and urinary excretion of salt and water. When salt retention is less actively stimulated, fecal loss of sodium on resin is greater and the diuresis in response to acidosis is more significant. Whether a given patient will respond must be determined by therapeutic trial.

Contraindications. Resins should not be used in severe renal disease because of the danger of marked acidosis and its attendant oliguria and azotemia Potassium and ammonium cycle resins are absolutely interdicted in acute renal failure and possible gain from the use of hydrogen and sodium cycle resins in removing potassium from the body must be balanced against the acidosis and edema which they induce. Ammonium cycle resins should not be employed in patients with severe liver insufficiency because of the possibility of ammonia toxicity. Adequate potassium intake must

reserves and are to be avoided if the patient has been overhydrated with saline early in the course of his disease.

Patients maintained on a low sodium diet, given daily doses of resins and subjected to repeated mercurial diuresis may develop the low salt syndrome, i.e., dilutional hyponatremia. This condition is discussed in Chapter XIX. When resins are given daily for long periods of time, hypocalcemia, muscle cramps and latent tetany may develop. Digitalis toxicity in the course of resin therapy is commonly associated with potassium depletion.

Dosage and Route of Administration. Resins are best given by mouth, but can be given per rectum in acute renal failure if nausea and vomiting make the oral route impractical. Resins are commonly given in amounts of 30 to 60 gm. per day in divided doses with meals. They are moderately unpleasant in taste and texture but can be adequately masked by suspension in fruit juices or tomato juice or by mixing in mashed potatoes or apple sauce. They may be given daily, or in interrupted courses of 4 days of drug, 3 days drug free.

A variety of preparations of carboxyl resins are available. Resodec is a mixture of two-thirds ammonium and one-third potassium cycle resin. Carboresin is a mixture of 88 per cent carboxyl resin and 12 per cent polyamine resin. Of the 88 per cent carboxyl resin, two-thirds is in the hydrogen cycle, one-third in the potassium cycle. Evidence that such amounts of polyamine resin significantly reduce acidosis is inconclusive. For potassium removal in acute renal failure, pure sodium cycle and pure hydrogen cycle resins are available.

Clinical Use of Cation Exchange Resins. Opinions differ as to the role of cation exchange resins in the therapy of edematous patients. A rather conservative view is that potent diuretics should be administered to effect the elimination of edema fluid. Thereafter resins and moderate dietary salt restriction may be employed to keep the patient edema free. In favorable cases, salt intake may be liberalized to such an extent that an easily prepared, nutritious and palatable diet is possible. Since resins induce a hyperchloremic metabolic acidosis, they potentiate the action of organomercurial diuretics. This can be considered as a useful by-product of resin

disturbances and clinical symptoms during prolonged exchange resin therapy in congesitve heart failure. *Am. J. Med.*, *14*:391, 1953.

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also be assured. Elevation of blood ammonia and potassium depletion are contributory factors in precipitation of liver coma.

SUMMARY

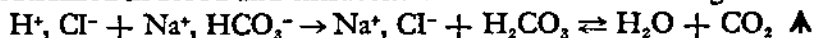
Cation exchange resins are macromolecular polyanionic lattices, insoluble and non-absorbable. When introduced in the digestive tract in hydrogen, ammonium and potassium cycles, they exchange these ions in variable degree for sodium present in the digestive secretions. At best their sodium binding capacity in the gut is low, and large doses must be administered to extract appreciable quantities of this ion. Hydrogen and ammonium cycle resins produce hyperchloremic metabolic acidosis, and since they preferentially bind potassium, tend to deplete body stores of this ion. Administration of one-third of the total resin dose in the potassium cycle in most instances protects against a negative potassium balance.

In favorable cases, the administration of resins abstracts sufficient sodium in the feces to permit some liberalization of intake without gain in weight. This somewhat simplifies preparation of a diet, and makes possible one of greater palatability and nutritive value. In some edematous patients, the acidosis induced by the resin causes primary diuresis and loss of weight. Resins, like other acidifying agents, potentiate mercurial diuresis. In some patients, maximally conserving sodium, resins abstract negligible quantities of this ion in the feces, instead abstract potassium. Adequacy of therapeutic response is largely dependent on degree of adrenal steroid stimulation of a colonic mechanism which reabsorbs sodium from the feces in exchange for potassium.

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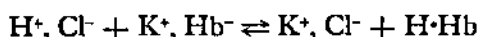
buffers contribute to the neutralization of strong acid in the body; the most familiar is bicarbonate. Hydrochloric acid is in part neutralized in blood and extracellular fluid in the following manner:



$$H^+, Cl^- + Na^+, HCO_3^- \rightarrow Na^+, Cl^- + H_2CO_3 \rightleftharpoons H_2O + CO_2 \uparrow$$

The net result is that a strong highly ionized acid, H^+, Cl^-, is converted into a weak un-ionized acid, H_2CO_3. Of equal importance, this weak acid is dehydrated to CO_2 and excreted by the lungs. The strong acid has completely disappeared, along with some fraction of the bicarbonate, and an equivalent amount of neutral sodium chloride has taken its place. Incidently pH decreases, but not in proportion to the fall in bicarbonate concentration, for breathing is stimulated. The administration of ammonium chloride therefore depresses plasma pH and bicarbonate concentration and elevates plasma chloride concentration, i.e., produces hyperchloremic metabolic acidosis.

A second buffer system of significance is hemoglobin. Hemoglobin exists in part as an ionized salt, potassium hemoglobinate, in part as a weak un-ionized acid, hemoglobin. Hydrochloric acid penetrates and is neutralized within erythrocytes in the manner described by the following equation:



$$H^+, Cl^- + K^+, Hb^- \rightleftharpoons K^+, Cl^- + H{\cdot}Hb$$

Again a strong acid has disappeared to be replaced by a weak acid. The reaction depicted by this equation is dependent on the general buffer properties of hemoglobin, not on its respiratory function in the transport of oxygen and carbon dioxide. To a slight extent the plasma proteins play a similar role, but since they are present in lower concentration than hemoglobin, their contribution is less significant.

It is a common misconception that the major part of an administered acid load is neutralized by blood buffers. Actually nothing could be further from the truth. As Van Slyke and Cullen pointed out many years ago and as Swan, Schwartz and others have recently emphasized, blood buffers neutralize but 15 to 20 per cent of an administered acid load; interstitial fluid buffers, largely bicarbonate, neutralize 30 to 35 per cent; and fully 50 per cent is neutralized by cellular buffers and by bone.

Chapter XI

ACIDIFYING AGENTS

AMMONIUM CHLORIDE

THE diuretic properties of acidifying salts were first described by Schultz in 1918. However, our present appreciation of the alterations in acid base balance and ionic structure of body fluids which these agents induce and which account for their diuretic action is based on the classic studies of Haldane, Gamble, Loeb, Keith and others. Ammonium chloride and nitrate, calcium chloride and nitrate, and hydrochloric acid have all been administered as acidifying agents. The nitrates have been discarded because they occasionally cause methemoglobinemia as a consequence of their reduction to nitrite in the gut. Calcium salts have been discarded because they frequently cause epigastric distress, abdominal discomfort, constipation, malaise, and muscle cramping. Ammonium chloride remains as the agent of choice, although cation exchange resins and acetazoleamide may also be employed to acidify the body fluids.

Ammonium chloride is rapidly absorbed in the gut, transported to the liver by the portal circulation and there converted to urea and hydrochloric acid. The net effect on acid base balance is the same as though an equivalent amount of hydrochloric acid had been ingested. The calcium ion of calcium chloride is poorly absorbed; most of a large oral dose is excreted in the feces as insoluble carbonate and phosphate. The chloride ion is readily absorbed in exchange for bicarbonate. The net effect again is equivalent to the ingestion of hydrochloric acid.

Neutralization of Acid in the Body. According to Van Slyke, the normal human body contains enough buffer to neutralize 1000 mEq. of hydrochloric acid before the pH of cells and extracellular fluid decreases to a level incompatible with life. A variety of

each day in divided doses. Chloride excretion rose sharply on the first day. Although the excretion of ammonia and titratable acid increased modestly, nearly all of the excess chloride in the urine was neutralized by sodium withdrawn from body reserves. During the subsequent 4 days of acid ingestion, progressively less and less

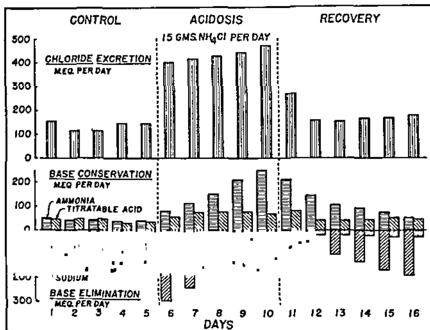


Fig 26 Effects of an acid load (15 gm per day of ammonium chloride) on the excretion of sodium, potassium, chloride, ammonia and titratable acid in normal man. (Drawn from data of O W Sartorius, J C Roemmelt, and R.F. Pitts· *J Clin. Invest*, 28 423, 1949)

sodium was sacrificed to neutralize chloride. Instead, on the second and third days, potassium, withdrawn from cellular stores, was utilized in increasing amounts. However, during the last 2 days the major fraction of the urinary chloride was eliminated in combination with ammonia. In fact, this mechanism permitted the achievement of acid base equilibrium and halted the loss of sodium and potassium by the fifth day of acid ingestion.

The loss of appreciable quantities of sodium and potassium from extracellular and cellular reserves is associated with the

Most cells, other than erythrocytes, can be described as effectively impermeable to chloride ions. Therefore, hydrochloric acid per se cannot penetrate to be neutralized by cellular buffers, i.e., by protein and organic phosphate complexes. However, cells are permeable to H^+ ions and to Na^+ and K^+ ions. Cells share their buffering powers with extracellular fluid by permitting the entrance of H^+ ions in exchange for equivalent numbers of Na^+ and K^+ ions which leave the cells. The chloride of hydrochloric acid remains extracellular.

$$2H^+ + K^+, Pr^- + Na^+, Org\ Po_4^- \rightleftharpoons H \cdot Pr + H \cdot Org\ Po_4 + Na^+ + K^+$$

Ammonium chloride therefore causes the loss of both potassium and sodium ions from cells and the gain of hydrogen ions. Accordingly the *pH* of the cell contents decreases. Since hydrogen ions bound to intracellular buffers are less ionized than were the sodium and potassium ions originally present, the osmotic pressure is reduced and water is lost by osmosis. According to Bergstrom and Wallace bone shares a part of its buffer capacity with extracellular fluid in much the same manner as do cells, giving up sodium ions in exchange for hydrogen ions.

Renal Compensations for Acid Loading. The study of Sartorius and Roemmelt, summarized in Figure 26, describes the alterations in renal excretion of ions in a normal individual over a five day period, during which 15 gm. of ammonium chloride were ingested each day. For the entire 16 day course of the experiment, the subject was maintained on a diet of constant composition with respect to electrolytes and calories. During the first 5 days, which constituted the control period, the subject excreted an average of 130 mEq of chloride, 125 mEq. of sodium, and 78 mEq. of potassium. Because the diet, like all normal diets, had an acid ash residue, 40 mEq. of ammonia and 40 mEq. of titratable acid were eliminated each day to balance the acid base budget. Ammonia and titratable acid are plotted upwards from the base line across the lower part of the chart to indicate that they represent cation conservation Sodium and potassium are plotted downward from the same base line to indicate cation loss.

During the second 5 day period, 15 gm. of ammonium chloride, equivalent to 280 mEq of hydrochloric acid, was administered

creasing quantities, until by the fifth day it neutralized all of the excess urinary anion. The ammonia excretory mechanism is the only truly compensatory one, in that it protects all cation reserves of the body. The potassium exchange mechanism, however, permits the participation of the large buffer reserves of cells in the homeostasis of body neutrality. The loss of ions from cellular and extracellular compartments is accompanied by a nearly equivalent loss of water. However, loss of water and decline in body weight are not strictly proportional to loss of ions, for plasma sodium concentration and total osmolality decrease somewhat.

The gradual increase in ammonia excretion under acid loading is a well documented observation of considerable significance. Because ammonia output increases slowly, body sodium is lost during the first few days of acid therapy. Because ammonia output rises to equal the acid load within 3 to 5 days, the diuretic response is brief. Nothing is gained by prolonged ammonium chloride therapy, the drug should be given in interrupted courses of 3 to 4 days, separated by equal or longer drug free intervals. The finding of rapid restoration of body sodium stores when ammonium chloride is withheld, emphasizes the fact that sodium intake must be severely restricted, if the losses sustained during acid loading are to be maintained in the drug free interval.

Davis and Yudkin, Rector, Seldin *et al.*, and Leonard and Orloff have shown that the gradual increase in rate of excretion of ammonia with acid loading is at least in part due to an adaptive increase in the activity of the enzyme, glutaminase, in the renal tubular cells. This enzyme catalyzes the deamidation of glutamine to glutamic acid; hence increases the rate of production of ammonia. The ammonia diffuses into acid urine where it is trapped as ammonium ion. As pointed out in Chapter IV, buffering of hydrogen ions by ammonia permits continued exchange of hydrogen for sodium. An increased rate of ammonia production obviously favors the salvage of sodium by distal tubules.

The stimulus for the adaptive increase in glutaminase activity might be either an increase in acidity of tubular cells or a reduction in body sodium stores. That the latter might be the more significant factor is suggested by the findings of Schwartz et al. They

excretion of nearly equivalent quantities of water and reduction in body weight. Normal individuals on such acidifying regimens lose a total of 1.5 to 3.0 Kg. of body weight before attaining acid base equilibrium. Usually some three-quarters of the fluid is withdrawn from the extracellular compartment and one-quarter from the cellular, although in the experiment described in Figure 26, losses were nearly equally distributed between the two compartments.

Net losses of sodium over a 5 day period have varied from 170 to over 300 mEq. in normal subjects. Potassium losses have varied from one third to half or more of sodium losses. The repair of depleted body reserves of sodium and potassium is illustrated in the last 6 days of the experiment shown in Figure 26. The excretion of cations decreased to very low levels during the first two days of recovery, although dietary intake remained the same. Anions were eliminated largely in combination with ammonia, while sodium and potassium were retained to replenish depleted extracellular and cellular reserves.

Mechanism of the Diuresis Induced by Acidifying Salts. The factors outlined above constitute the basis for an explanation of the diuretic action of ammonium chloride. The hydrochloric acid formed in the liver is in part neutralized by bicarbonate of blood and interstitial fluid. Bicarbonate is converted to chloride and the carbon dioxide is expelled by the lungs. In the experiment described in Figure 26 plasma chloride increased from 110 to 123 mEq per liter, plasma bicarbonate decreased from 27 to 15 mEq. per liter. The sum of the two anions remained essentially constant. The load of chloride delivered into the tubules in the glomerular filtrate increased as a result of the increase in plasma concentration. The filtered load of bicarbonate decreased. Within limits the capacities of the tubules to reabsorb chloride and bicarbonate vary reciprocally, but the reciprocity is not perfect. Although chloride reabsorption increased, some of that which was filtered escaped into the urine. On the first day sodium was sacrificed to neutralize urinary chloride. On the second and third days increasing quantities of potassium were excreted in exchange for sodium, thereby protecting sodium reserves from further depletion. From the first day onward, ammonia was substituted for sodium in steadily in-

though severely depleted of sodium. The glutaminase activity of their renal tubules is probably high. Accordingly, when ammonium chloride is administered, the chloride is largely excreted in combination with ammonia and potassium; little sodium is lost. No doubt low glomerular filtration rate and excessive salt retaining hormone production contribute to failure of diuresis in edematous patients actively conserving sodium.

Dose and Route of Administration. Ammonium chloride is administered orally in doses of 2 to 5 gm., 3 times a day for not longer than 4 days. Three days to a week are allowed to elapse between courses. When given to potentiate mercurial diuresis, the drug is given for 3 to 4 days, and on the last day, 2 ml. of a mercurial diuretic is given intramuscularly. Although it is commonly done, ammonium chloride should not be given as enteric coated tablets. When so administered, absorption is completely unpredictable, and usually inadequate. The taste of ammonium chloride is unpleasant and difficult to mask; syrup of raspberry is probably the most effective disguise. The drug should not be administered in a solution stronger than 2.5 per cent because of gastric irritation. It can be given in uncoated or gelatin coated tablets or in gelatin capsules along with ample water. It should be taken immediately before meals to minimize gastric discomfort. Ammonium chloride can be given intravenously in 1 to 2 per cent solution, made up in 5 per cent glucose. It must be given very slowly to avoid severe toxic reactions, at a rate not in excess of 2 gm. per hr. Under no circumstance should it be administered in such fashion to a patient with cirrhosis or evidence of hepatic dysfunction. Intravenous ammonium chloride is usually employed only in the treatment of severe metabolic alkalosis with vomiting, not for its diuretic effect.

Toxicity. Symptoms of gastrointestinal irritation are common, including epigastric distress, anorexia, nausea and vomiting. Symptoms of acidosis, mild in character, are to be expected if the dose of ammonium chloride is adequate. These include barely noticeable hyperventilation at rest but definite exertional dyspnea. Mild weakness and lassitude are common complaints. However, in the presence of severely impaired renal function, a fulminating type of

observed that the infusion of neutral sodium sulfate in normal subjects increases the acidity of the urine and the output of ammonia. When their subjects were depleted of sodium over a period of several days and sodium sulfate was then infused, the urine became intensely acid and far greater quantities of ammonia were eliminated. Presumably sodium depletion per se caused an adaptive increase in enzyme activity for no apparent change in acid base balance occurred as a result of sodium depletion. Enhanced exchange of hydrogen for sodium and greater production of ammonia permitted salvage of more sodium and a more rapid restoration of depleted sodium stores. However, intracellular acidity may well be a significant factor under other circumstances.

Clinical Use of Ammonium Chloride. Ammonium chloride is generally regarded as a mild diuretic in its own right. As pointed out above, therapeutic amounts produce a weight loss of 1.5 to 3.0 Kg. in normal subjects. In patients who respond favorably, the diuresis may be far more significant because of the availability of greater reserves of extracellular fluid. Ammonium chloride is a significant element of the Schemm regimen for the treatment of edema, which includes salt restriction, acidification, and the forcing of fluids. It plays an adjuvant role in diuretic therapy with osmotic diuretics and with chlorothiazide; in the latter instance it corrects the mild alkalosis induced by the primary diuretic drug. It is most widely used for its highly significant potentiation of mercurial diuresis (*cf.* Chapter XVI), and to correct the alkalosis which commonly results from intensive therapy with mercurial diuretics.

Ammonium chloride is perhaps ideally suited as a primary diuretic to overcome the slight tendency for fluid and salt retention which so discourages patients on a low caloric weight reducing regimen. The fact that it is apt to produce some nausea is an asset under these circumstances.

Frankly edematous patients, actively retaining sodium, rarely respond in satisfactory fashion to ammonium chloride alone, or else exhibit diuresis on the first day and become refractory thereafter. The findings of Schwartz *et al.*, alluded to above, explain this failure to respond. Such patients, although edematous, behave as

proper fashion, these two agents are the most powerful diuretics available to the physician today.

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acidosis develops with Kussmaul breathing, prostration, confusion, coma, azotemia and oliguria. When patients with moderate impairment of renal function are maintained on daily doses of ammonium chloride over long periods of time, such a picture may develop gradually. Since, as pointed out above, continuous therapy with ammonium chloride serves no useful purpose; a regimen which results in severe chronic acidosis cannot be too heartily condemned. Depletion of body stores of postassium may likewise result from prolonged administration of ammonium chloride, especially if anorexia prevents adequate dietary replacement. Dietary supplements of potassium are advisable. Signs of ammonia toxicity, including weakness, apathy, drowsiness, confusion and a coarse flapping tremor may develop in cirrhotic patients with hepatic insufficiency.

Contraindications. Ammonium chloride should not be given to patients with severely impaired renal function because of the danger of severe acidosis. The drug should not be given to patients with evidence of liver insufficiency because of the danger of ammonia toxicity and liver coma.

SUMMARY

Ammonium chloride induces hyperchloremic metabolic acidosis. Because plasma concentration increases, the filtered load of chloride delivered into the renal tubules increases. Although tubular reabsorption is enhanced, the excess load of chloride is not entirely removed from the urine. The chloride excreted on the first day is neutralized almost entirely by sodium; on the second and third days, it is neutralized to a significant extent by potassium. By the fifth day urinary chloride is almost entirely neutralized by ammonia. Loss of sodium during the first few days is accompanied by loss of extracellular water; loss of potassium, by loss of cell water. Because the diuretic response is brief, ammonium chloride should be administered in interrupted courses of short duration, separated by drug free intervals. Ammonium chloride is at best a mild diuretic and is usually employed as an adjuvant in therapy with more potent compounds. It serves its most useful purpose in potentiating the action of mercurial diuretics. Used together in

cortical secretion of aldosterone or the competitive inhibition of renal salt retention by the administration of a steroid which, although it had no salt retaining properties itself, would bind to renal tubular receptor sites and displace aldosterone. Amphenone B is a compound having the first action, namely inhibition of aldosterone secretion. Compounds designated as SC5233 and SC8109 are examples of the second type which apparently displace aldosterone competitively from the renal tubular receptor sites Just how ACTH, cortisone, prednisone, etc. exert their favorable actions is less certain at the moment.

Indications for Steroid Therapy of Edema. Widespread clinical experience justifies the statement that one or another of the following, ACTH, cortisone, prednisone or prednisolone, is the therapeutic agent of choice in the juvenile form of socalled "genuine lipoid nephrosis" and in the nephrotic stage of glomerulonephritis if renal function is adequate. Unfortunately, steroid therapy of nephrosis is not always successful. and other diuretic procedures have their place. Prednisone and prednisolone would seem at least theoretically preferable to ACTH and cortisone because of lesser salt retaining activity. The use of these agents in cardiac and cirrhotic edema and indeed in the nephrotic syndrome as well should be restricted to those patients who can be kept under close surveillance, maintained on a rigidly low salt intake, and observed continuously for complications of steroid therapy. Recent reports suggest that these steroids are especially useful in facilitating the elimination of water in the condition of hyponatremia which not infrequently develops following paracentesis or vigorous diuretic therapy with mercurial compounds. They also have been described as potentiating the action of acetazoleamide and mercurial diuretics in patients resistant to those agents.

Amphenone is too toxic for general therapeutic use but is especially interesting because of the light it has shed on the role of hypersecretion of steroids in primary diseases of the adrenal as well as in edema It may well be the harbinger of diuretic agents of the future. The compounds SC5233 and SC8109 are experimental drugs, do not seem especially potent, but are promising leads in a search for a new approach to diuretic therapy.

Chapter XII

STEROID AND ANTISTEROID THERAPY

ACTH and cortisone were first used in the treatment of nephrosis by Farnsworth and by Luetscher just a decade ago. Some 5 years later Schemm, Camara, Heidorn, Cattan and Vesin, and others reported the surprising observation that these agents, known under most circumstances to cause sodium retention, frequently induced diuresis in patients with cardiac and cirrhotic edema as well as in those with the nephrotic syndrome. More recently two synthetic steroids, prednisone and prednisolone, related respectively to cortisone and to hydrocortisone, but having less sodium-retaining potentialities, have been employed in the treatment of a variety of edematous states by Muller, Mach, Landan, Riemer, and others.

The fact that the urinary excretion of salt retaining steroid, identified in more recent studies as aldosterone, is increased in patients with nephrotic, preeclamptic and cardiac edema and in those with cirrhosis and ascites has been demonstrated by Deming, Luetscher, Chart, Singer, Venning, and others. The additional observation that the sodium content of the urine, feces, sweat and saliva is reduced in edematous patients suggests that the concentration of aldosterone in the body fluids is high, due either to increased production or decreased destruction, and that overabundance of this steroid is a significant factor in the pathogenesis of edema and ascites. Davis has shown in the dog with experimental ascites and Marson and Werk et al have shown in patients with cirrhosis and ascites that bilateral adrenalectomy reduces sodium and water retention and peritoneal transudation. The difficulties of hormonal control of bilaterally adrenalectomized patients makes this something less than an ideal therapeutic procedure. A more rational type of treatment would be the inhibition of adrenal

hormone synthesis in the adrenal cortex, not by altering its rate of metabolism. While most of the above findings demonstrate interference with synthesis of glucocorticoids, it is evident from Rosenfeld and Bascom's observations that certain synthetic operations basic to production of aldosterone are also reversibly inhibited.

Renold et al observed that the exhibition of amphenone to a patient with a metastasizing adrenal carcinoma with Cushing's syndrome led to a cessation of urinary excretion of aldosterone and to a brisk diuresis of salt and water. McCullagh and Tretbar administered as much as 9.25 gm. of amphenone per day to patients with Cushing's syndrome with minimal signs of toxicity. Others, however, have noted that effective doses cause gastrointestinal disturbances, including nausea, vomiting, diarrhea and abdominal distension, methemoglobinemia, bone marrow depression, cutaneous eruptions and thyroid enlargement. In view of the multiplicity and potentially serious nature of the complications of therapy, amphenone does not seem to be a satisfactory drug for general clinical use.

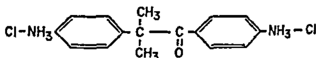
NATURAL AND SYNTHETIC CORTICOSTEROIDS

General Nature and Properties of Adrenal Steroids. Approximately 30 steroids have been isolated from the adrenal cortex, most no doubt are either chemical intermediates in the processes by which the gland synthesizes its relatively few secretory products or artifacts formed in the process of chemical extraction. Intensive study of the chemistry of these compounds was begun about 25 years ago by Kendall, Pfiffner, Reichstein, Wintersteiner, and their respective associates. Their studies, which led through separation, identification of structure, to synthesis, constitute a chemical advance of tremendous significance to biology and medicine.

Figure 27 illustrates the structure of 8 steroids of significance to our discussion. All are related to allopregnane, the structure of which is shown with the several carbon atoms numbered. Those steroids with adrenal cortical biological activity of one sort or another have the following structural characteristics: (1) a double bond between carbons 4 and 5, i.e. unsaturation of ring A; (2) a

ALDOSTERONE ANTISECRETORY AGENTS-AMPHENONE

Amphenone (1,2-bis(p-aminophenyl) - 2 - methyl propanone - 1 dihydrochloride) is one of a series of desoxybenzoins synthesized by Allen and Corwin in 1950, and has the following structure.



It was observed by Hertz and his associates to produce a marked enlargement of the adrenals of rats, associated with increased deposition of lipid materials. To a lesser extent it causes enlargement of the thyroid. In the hypophysectomized animal, neither the adrenals nor the thyroid are enlarged by amphenone. Furthermore, in the normal rat, cortisone prevents enlargement of the adrenals, and thyroid feeding prevents the development of goitre when amphenone is given.

One may infer from these facts that enlargement of the adrenals is due to enhanced ACTH secretion, and that goitre is due to enhanced TSH (thyroid stimulating hormone) secretion. The trophic hormones of the hypophysis are secreted in increased amounts because the production of adrenal steroids and thyroid hormone is suppressed. The normal feedback mechanisms, by which cortical and thyroid hormones suppress the output of trophic hormones by the hypophysis, is lost; ACTH and TSH are therefore secreted in increased amounts, leading to enlargement of the adrenals and to goitre. Hume and Nelson observed that the administration of ACTH to acutely hypophysectomized animals greatly increases the production of adrenal steroids, an action which is suppressed by amphenone. Dorfman noted that the production of 17-hydroxy corticoids in vitro by adrenal slices is inhibited by amphenone, and Rosenfeld and Bascom observed that the drug interferes with the synthesis of hydrocortisone, cortisone and corticosterone by the surviving perfused adrenal gland. Finally, Peterson, Hertz, and Lubs, utilizing isotopically tagged hydrocortisone and cortisone, have shown that amphenone exerts its effect in man by suppressing

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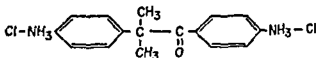
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Aldosterone was isolated in 1953 from the amorphous fraction of the adrenal cortex and its structure was determined in 1954 by Simpson, Tait, Wettstein, Euw, and Reichstein. The steroid was synthesized in 1955 by Wettstein and colleagues. It can be described chemically as 18-aldehydocorticosterone and exists in the body as an equilibrium mixture of the aldehydo and hemiacetal forms, largely the latter. Most of the sodium retaining hormone activity present in adrenal venous blood is due to aldosterone, a compound variously described as 25 to 30 times as potent as desoxycorticosterone in stimulating sodium reabsorption and potassium secretion. As was pointed out in Chapter V, its rate of secretion is relatively independent of hypophyseal trophic hormones and is possibly under the control of a hypothalamic humoral regulatory mechanism. This mechanism is activated in response to a diminution in extracellular volume or some derivative of volume, such as pressure or flow.

Aldosterone is metabolized by the liver as are so many steroid hormones. A minute fraction, probably not more than 1 or 2 per cent of the total output of the glands, is excreted in the urine. Most of the urinary hormone is excreted in a conjugated form, having no biological activity, but hydrolyzable at pH 1.0 to the parent active compound The normal individual on a liberal salt intake excretes from 1 to 4 μgm. per day; the cardiac in severe congestive failure may excrete upwards of 50 μgm. per day; and the decompensated cirrhotic, actively accumulating ascites, may excrete from 50 to 300 or more μgm. per day. While there is little doubt that aldosterone secretion is greatly increased under conditions of active salt retention, there is no way to partion increased urinary excretion between increased production and decreased tissue and hepatic destruction. From the results of Yates et al one may reasonably infer that the cirrhotic or congested liver metabolizes the hormone less effectively than does the normal.

COMPETITIVE INHIBITION OF MINERALOCORTICOID ACTIVITY

Two synthetic steroids, designated respectively as SC5233 and SC8109, exhibit the intriguing property of reversibly inhibiting

carbonyl oxygen on C_3; (3) a 2 carbon side chain attached to C_{17} of ring D with a ketone oxygen on C_{20} of this side chain and a hydroxyl group on C_{21} (α-ketol side chain). (4) Compounds with oxygen atoms on both C_{11} and C_{17} such as cortisone, hydrocortisone, and the synthetic analogues, prednisone and prednisolone

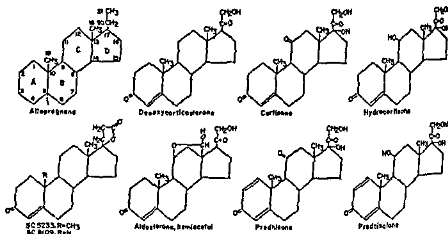


Fig. 27. Structure of natural and synthetic steroids which have an effect on the renal tubular reabsorption and excretion of ions

have predominantly glucocorticoid activity with lesser effects on electrolyte metabolism. Aldosterone is an obvious exception to this rule. (5) Further unsaturation of ring A by a double bond between carbons 1 and 2, as in prednisone and prednisolone, reduces even more the effects of these steroids on electrolyte metabolism.

Mineralocorticoids of the Adrenal Gland. Desoxycorticosterone was first synthesized by Steiger and Reichstein in 1937, prior to its isolation from the gland a year later by Reichstein and Euw. It is present in the adrenal cortex in minute amounts and for a time was considered to be strictly an intermediate in the synthesis of other steroids. However, it has recently been identified in adrenal venous blood and must be considered a normal glandular product. This steroid is almost devoid of glucocorticoid activity but, as was shown by Loeb and others some 20 years ago, has a profound effect on electrolyte metabolism, promoting the renal reabsorption of sodium and the secretion of potassium.

caused loss of sodium, retention of potassium, decrease in plasma bicarbonate and restoration of a normal electrocardiographic pattern. It is especially significant that these changes occurred in the presence of increased urinary excretion of aldosterone. Pre-drug excretion of aldosterone averaged 44 μgm. per day, during drug therapy, 89 μgm. per day, and on cessation of drug treatment, 44 μgm. per day. These findings rule out any feedback action whereby SC8109 might inhibit the hypothalamic controlling center and reduce secretion of aldosterone; they likewise rule out any interference with production of or any stimulation of destruction of aldosterone as an explanation of activity of the synthetic steroid.

USE OF NATURAL AND SYNTHETIC CORTICOSTEROIDS IN THE TREATMENT OF EDEMA

ACTH and Cortisone in Nephrotic Edema. The nephrotic syndrome is characterized by proteinuria, hypoproteinemia, elevation of blood lipids and variable edema The form of the disease most common in childhood, namely that which has an insidious onset, without anticedent hematuria and without associated azotemia and hypertension, runs a highly variable course. Without specific therapy, the disease is marked by chronic fluctuating edema, frequently resistant to conventional diuretic therapy. Before the advent of antibiotics one in three died of infections, to which they are peculiarly susceptible, another recovered spontaneously and completely, and the third progressed to chronic nephritis and died in uremia Now that control of infection is possible, the prognosis is somewhat brighter, and as Luetscher points out, treatment should be primarily directed to survival. Attention should be directed to prevention and control of intercurrent infection, to maintenance of nutrition, to prevention of cardiac failure and to control of renal insufficiency and massive edema. Salt free concentrated serum albumin or hyperoncotic dextran administered intravenously (see Chapter VIII) and/or ACTH and adrenal steroids are useful in the control of edema, although there is no clear-cut evidence that they alter the ultimate course of the disease.

Rationale of Use of Steroids in the Nephrotic State. A number of clinicians have noted the association of recovery from a variety

the increased tubular reabsorption of sodium and increased secretion of potassium induced by desoxycorticosterone and by aldosterone. The structure of these steroids is shown in Figure. 27. They differ from the usual adrenal steroids in having a propionic acid lactone ring attached to the C17 site, in place of the customary α-ketol side chain and hydroxyl group. Like desoxycorticosterone these steroids are 11-desoxy compounds. SC8109 differs from SC5233 in having a hydrogen instead of a methyl group (C19) attached to C_{10}. Both are known as spirolactones.

Kagawa and his associates have shown that these synthetic steroids have little effect on the excretion of sodium and potassium in the normal rat. Aldosterone, in contrast, in an amount of 0.96 μgm. markedly enhances sodium reabsorption and potassium secretion. However, the simultaneous administration of 1.2 to 1.3 mg. of SC5233 or SC8109 blocks the action of aldosterone. These steroids likewise block the renal tubular actions of desoxycorticosterone. Kagawa maintains that aldosterone and desoxycorticosterone compete with the synthetic steroids for common receptor sites on tubular cells, that this competition is reversible, and that it can be described in terms of the mass law, the affinity of the natural steroids for the receptor sites being many times that of the synthetic ones.

Liddle has carried these observations over to man, showing that SC5233 has no effect on sodium excretion in normal man on high salt intake, nor in the untreated Addisonian patient. When the patient with Addison's disease is controlled with desoxycorticosterone, SC5233 is natriuretic. Furthermore, the steroid is natriuretic in normal individuals on a low salt intake, under which condition aldosterone production is relatively great. When given to patients in congestive failure, SC5233 produces a modest diuresis of salt and water and a loss of body weight.

Salassa, Mattox and Power have confirmed the basic elements of these views in a study on a patient with primary hyperaldosteronism due to a cortical adenoma (proven at subsequent operation). The patient presented with the typical picture of polyuria, alkalosis, severe hypertension and electrocardiographic evidence of hypokalemia. The administration of 1.9 gm. per day of SC8109

and more prolonged therapy. Some patients who do not respond to steroids either during or after therapy may exhibit diuresis when treated with concentrated salt poor albumin or when given diuretics during a course of hormone treatment.

Mechanism of Diuretic Action of Steroids in Nephrosis. All investigators agree that accompanying or preceding loss of edema, there occurs a significant increase in glomerular filtration rate; an increase in serum sodium concentration, a marked decrease in protein excretion, an increase in plasma protein concentration and a decrease in the rate of urinary excretion of salt retaining steroids. Just which of these factors are causes and which are results of diuresis is by no means clear. There exists, according to Gaunt, an antagonism between antidiuretic hormone and the 11, 17-oxysteroids which finds its negative expression in the very slow rate of excretion of a water load by the adrenalectomized animal or by the patient with Addison's disease. The water diuresis which commonly precedes the sodium diuresis in the nephrotic patient under steroid treatment and which is no doubt responsible for the increase in serum sodium concentration, possibly results from the antagonism of the water retaining properties of ADH by the polyuric action of 11, 17-oxysteroids. In terms of the hypothesis of Wirz and Sawyer based on Ussing's work, ADH dilates pores in the distal tubules and collecting ducts, permitting the osmotic return of water to the blood stream and the formation of a small volume of concentrated urine (see Chapters IV and V); in contrast, 11, 17 oxysteroids constrict the pores, preventing reabsorption of water and leading to the formation of dilute urine.

Lauson et al have shown that ACTH abruptly decreases protein excretion due to a reduction in the abnormally high permeability of the glomerular capillaries to albumin. Associated with diminished protein excretion and elimination of excess water, serum protein concentration rises, a factor which promotes further transfer of fluid from the interstitial to the vascular compartments. What may be another manifestation of reparative processes is increased glomerular filtration rate. Eder et al logically point out that an increase in the filtered load of sodium and water serves to correct the tubular preponderance which led initially to edema formation.

of acute infections and remission of the nephrotic state. Measles has been most frequently described as effective and, in the past, some have advocated induction of this disease as a therapeutic measure. Typhoid vaccine and other forms of fever therapy have also been used to induce remission. The stress of acute infections associated with high fever probably calls forth an intense adrenal response. Increased secretion of adrenal steroids might well be the link between infectious disease and remission. Many believe that the initial renal insult in nephrosis is immunogenic in nature. The dramatic effects of ACTH and cortisone in suppressing antigen-antibody reactions suggests that this action may underlie the *favorable response to adrenal steroids.* No doubt curiosity as to the possible effects of new and dramatically active agents on a disease of unknown etiology must have played some role in the initial trials of ACTH and cortisone in nephrosis by Farnsworth and Luetscher. Many, including Thorn, Barnett, Lauson, Riley, and others have confirmed the fact that diuresis and remission of the disease frequently occurs during or within a day or two after cessation of treatment with ACTH and cortisone.

Nature of the Diuretic Response in Nephrosis. The early studies of Luetscher and Thorn indicated that ACTH was more effective than cortisone in inducing diuresis and remission of disease. Subsequently others have claimed that cortisone and prednisone are equally effective. In any event when any one of the three hormones is given in adequate dosage over a period of 10 to14 days, one of three results may be expected. (1) Diuresis begins on the second to the sixth day, initially as a water diuresis and then associated with increasing serum sodium concentration, it continues as a sodium diuresis. When therapy is stopped, a more intense diuresis occurs, resulting in complete loss of edema. (2) No diuresis occurs during steroid therapy, instead begins when hormone administration is terminated. (3) No clinical improvement results during or after therapy. Remissions vary in length from a few days to many months; indeed all evidence of disease disappears in some. In the event of exacerbation of the nephrotic state, patients are responsive to subsequent courses of therapy. Luetscher advises under these circumstances higher steroid dosage

and more prolonged therapy. Some patients who do not respond to steroids either during or after therapy may exhibit diuresis when treated with concentrated salt poor albumin or when given diuretics during a course of hormone treatment.

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Filtration rate has been observed to increase as much as 150 per cent coincident with diuresis. According to Barnett et al, repeated courses of therapy may progressively increase glomerular filtration rate to or toward normal, even in patients with long standing disease and marked functional impairment. Tubular secretory capacity for para-aminohippurate and tubular reabsorptive capacity for glucose likewise improve under steroid therapy.

Early studies of Forsham and of Thorn suggest an antagonism between 11, 17-oxysteroids and desoxycorticosterone-like salt retaining hormones. Were this antagonism exerted at the target organ, the renal tubular cell, it would explain natriuresis during therapy with either cortisone or ACTH. However, it would not explain the reduced renal excretion of salt retaining steroids observed by Luetscher and others to accompany effective diuresis. If one accepts the fact that a fraction of aldosterone secretion is under pituitary control, then cortisone but not ACTH therapy would be expected to depress its secretion (i.e., feedback depression). Renal tubular fatigue, reduced sensitivity of the tubules to salt retaining hormone, and depression of secretion of aldosterone by cortisone and ACTH have all been suggested as possible explanations of depressed tubular reabsorption of sodium. If, as Farrell maintains, aldosterone secretion is controlled by a hormone produced in the hypothalamus, cortisone or similar steroids, secreted in response to ACTH, might depress the formation of the regulatory neurohumor.

Dose and Route of Administration. From 12.5 to 25 mg. of ACTH is given intramuscularly every 6 hr. for a total daily dose of 50 to 100 mg. Cortisone or prednisone is given orally in four equal doses at 6 hr. intervals; the total daily dose of cortisone is 200 mg; that of prednisone, 50 mg. Treatment is continued for 10 to 14 days and the drug is then abruptly withdrawn. If diuresis does not result either during or on cessation of therapy, the course may be repeated and after a few days of treatment, acetazoleamide, a mercurial diuretic, or no doubt other diuretics as well may be given. The patient ordinarily refractory to such diuretics, may respond well while on steroid therapy. During steroid therapy, dietary sodium intake must be rigidly restricted to between 10 and

30 mEq. per day (0.5 to 1.5 gm. NaCl per day) to avoid excessive fluid retention, and the possible complications of hypertension, congestive failure, pulmonary edema and convulsions.

*Effects of ACTH, Cortisone, Prednisone, etc., in Other Edematous States.*It is difficult to conceive that administration of steroids which are at least modestly salt retaining could exert a favorable effect on fluid and electrolyte balance in patients with congestive failure, with cirrhosis and ascites, or with pre-eclampsia. Indeed this conceptual hurdle probably accounts for the fact that steroid therapy of edema in these diseases was delayed some 5 years after its introduction in the treatment of nephrosis. In retrospect, one can provide a rationale for the use of corticosteroids on the basis of promotion of water diuresis by 11, 17 oxysteroids and a possible antagonism of the renal tubular effects of mineralocorticoids by glucocorticoids suggested by Thorn, Jailer and others.

ACTH was first used by Schemm and Camara in 1954 to treat mercury resistant, hyponatremic patients in severe congestive failure. Their regimen includes strict dietary salt restriction (9 to 30 mEq. per day), an acid ash diet plus 1.5 to 4.0 gm. of ammonium chloride per day, a bountiful fluid intake (2500 to 4000 ml. per day), adequate digitalization, bed rest, sedation, oxygen if needed, and ACTH, 15 to 25 mg. q. 6 hr. Frequently on the third to the sixth day profuse water diuresis occurs, followed by salt diuresis and loss of weight. If no diuresis occurs by the sixth day, 2 ml. of Thiomerin is given intramuscularly. Often a profound diuresis and loss of weight ensue in patients previously unresponsive to diuretic therapy. Camara and Schemm maintain that an adequate diuretic response can be obtained in 80 per cent of patients with hopeless, terminal, mercury resistant congestive failure.

More recently Cattan and Vesin, Riemer, Muller, Fabre and others have administered prednisone and prednisolone, steroids with only minor salt retaining potentialities to patients with cirrhosis and ascites, congestive failure, pre-eclampsia and nephrosis. The response has been observed to be favorable in a high proportion of cases and essentially similar to that described above, namely (1) diuresis during therapy, (2) diuresis on discontinua-

tion of therapy, (3) return of sensitivity to diuretic therapy in previously resistant patients. The response that has been most frequently observed has been increased sensitivity to mercurials and carbonic anhydrase inhibitors.

The Mechanism of Action of Steroids in Edemas Other Than Nephrotic is mysterious to say the least. Indeed the very existence of diuretic activity in patients with congestive failure or cirrhosis with ascites renders suspect certain of the theories advanced above in explanation of diuretic action of steroids in nephrosis. There is no dearth of hypotheses, yet no one of them is adequately supported by experimental fact. The following constitute a representative sample: (1) antagonism of water retention due to ADH by adrenal corticoids, an action considered to be of special significance in the correction of hyponatremia; (2) increase in glomerular filtration rate, favoring spontaneous diuresis and/or a return of sensitivity to diuretics; (3) inhibition of aldosterone production; (4) increase in vasomotion of the terminal vascular bed, favoring return of fluid from the interstitial to the vascular compartments, (5) acceleration of activity of the tissue spreading factor which again favors return of fluid to the circulation, (6) decrease of permeability of glomerular and hepatic capillaries to protein; (7) an alteration of the "set" of the volume regulatory mechanism; (8) antagonism at the target organ (kidney) of mineralocorticoids by glucocorticoids.

A number of investigators have observed an increase in glomerular filtration rate in edematous patients during steroid therapy. While, to the author, it seems evident that any increase in filtration will tend to reduce the glomerulo-tubular imbalance which causes fluid retention, it is interesting to see how frequently and vociferously this is denied. Those who have measured aldosterone excretion have found it decreased under steroid therapy. Muller, however, has added the confusing but significant point that when Diamox or mercurials alone are administered to edematous patients, diuresis is accompanied by increased aldosterone excretion. When these same drugs are given to patients under prednisone therapy, diuresis is accompanied by decreased aldosterone secretion. While increased aldosterone secretion in response to potent diuretics is

easy to explain as a compensatory response to reduction of extracellular volume in a patient with an unaltered basic drive to retain salt, it is difficult to account for the opposite response with combined steroid and diuretic therapy. It implies that steroids correct the basic abnormality of congestive failure or cirrhosis (hard to believe), or that they alter the "set" of the volume control center (no evidence).

Dose and Route of Administration are the same in cardiac, cirrhotic and preeclamptic edema as in nephrotic edema described above. The major beneficial result is frequently increased sensitivity to more conventional diuretic agents in patients who previously responded poorly.

Complications of Steroid Therapy. One of the major hazards of steroid therapy is excessive retention of salt and water and precipitation of pulmonary edema. This may be avoided by strict limitation of salt intake (ideally to 10 mEq. of sodium per day), provision of an acid ash diet and administration of ammonium chloride. Despite precautionary measures some gain in weight can be expected initially. Hypertension is a complication, at least in part related to fluid retention. Other hazards include activation of peptic ulcer and hemorrhage from esophageal varices. For these reasons it is advisable to perform daily benzidene tests on the stool. Suppression of general systemic reaction and local tissue reaction to infection and irritation may mask serious acute infectious disease and render silent perforation of an ulcer.

SUMMARY

ACTH, cortisone, prednisone, and prednisolone have recently been employed in the treatment of edema and ascites in congestive failure, cirrhosis and pre-eclampsia in much the same fashion and with much the same result noted in their earlier use in the nephrotic state. They may be employed in the treatment of acute dilutional hyponatremia, in which condition they induce first a water diuresis which may be followed by a sodium diuresis when the hyponatremia is corrected. They also frequently restore responsiveness to mercurial diuretics and carbonic anhydrase inhibitors in patients who have become resistant to these drugs.

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The mechanism of their diuretic action is by no means clear. It may be that they induce water diuresis by antagonizing the action of ADH on the renal tubule. They may likewise antagonize the salt retaining effects of aldosterone. They usually cause an increase in filtration rate, a factor of some significance both in their primary diuretic action and in their potentiation of other diuretics. They may well act peripherally by altering capillary permeability, the distribution of fluid between capillaries and tissue interstices and the ion equilibria between cells and interstitial fluid.

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Chapter XIII

WATER IN DIURETIC THERAPY

THE importance of reducing sodium intake in the treatment of edema was increasingly appreciated over the first few decades of the present century. Today sodium restriction is accepted as a necessary procedure, limited mainly by the practicalities of providing a nutrious and reasonably appetizing diet. There is less general agreement concerning optimum water intake, and recommendations range from the archaic and inhumane view that fluids should be drastically restricted to the opposite extreme that fluids should be forced to the extent of 5 to 10 liters per day. Most clinicians favor a middle course, allowing moderate or ad libitum intake. Schroeder, Bridges, Crutchfield and many others have shown that restriction of sodium intake to a level below urinary output results in decrease of edema independent of the amount of water ingested. Furthermore, many have observed that digitalization, bed rest and other diuretic procedures cause weight loss when patients are taking liberal quantities of fluids but are ineffective when fluids are rigidly restricted

Sir Thomas Witherly's remarks[16] in a report before the Royal College of Physicians in 1690 are illuminating in this respect. "A Wine-Cooper fell into a Dropsy which resisted all the usual Methods. This Man was prodigiously swell'd, Belly, Back, Thighs and Legs. Being past all Hopes and having on him an inextinguishable Thirst, he was permitted to drink 14 Quarts of Water in about 10 hours, and in all that Time made not one Drop of Urine. Soon after he began to piss, and he drank on, 4 or 5 Quarts daily, and so recovered.—That Water should expel Water is a Miracle beyond any of St. Winifred's. Now no Man in his Senses would have prescribed such a water-course to cure a Dropsy, which

[16]Quoted from F. R. Schemm *Ann. Int. Med., 21:*937-976, 1944

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dextrose from 1 to 6 times per day. At first he observes no diuresis, a gain in weight and a visible increase in edema. Persistence is rewarded by diuresis and clearing of edema.

Vital elements of the Schemm regimen are rigid dietary sodium restriction, ideally to 9 mEq. per day; an acid ash diet supplemented with 1.5 to 4 0 gm. of ammonium chloride; bed rest; and digitalization, sedation, and oxygen for the patient in congestive failure, if needed. No doubt a number of the failures of those who have tried this regimen, have been due to inadequate restriction of sodium intake and/or inadequate attention to other elements of the regimen. However, acute dilutional hyponatremia has frequently resulted from the inadequate diuretic response of severely ill and edematous patients. Most who have tried the Schemm regimen have been less impressed with its efficacy as a primary therapeutic procedure than has he. The reason for discussing it is to emphasize once again that dehydration by fluid restriction defeats rather than promotes therapy of edema.

Mechanism of Salt Loss in Water Diuresis. Recent studies of Leaf, Weston, Wrong and their respective associates demonstrate clearly that salt loss can be induced in normal subjects by hyperhydration and that the magnitude of the loss is related primarily to the degree of expansion of volume of body water. They observed that subjects given daily doses of pitressin tannate in oil to induce antidiuresis and loaded judiciously with water, retain that water, develop an acute dilutional hyponatremia, expand cellular, interstitial and plasma stores of water, and after some 6 to 12 hr. exhibit a significant sodium diuresis. From 70 to 370 mEq. of sodium may be lost per day. When the administration of pitressin is stopped, water diuresis ensues over the succeeding 24 to 48 hr. and at its end, weight is reduced in proportion to the sum of the daily losses of sodium. Leaf maintains that sodium loss can be reduced by the coadministration of ACTH. In a similar vein, Wrong maintains that the delay of 6 to 12 hr. in the onset of saluresis after the imposition of a positive water load represents the time required for the relatively slow hepatic metabolism of circulating aldosterone.

shows how little we know of Nature and the great Uncertainty of our Art."

That water can expel water was observed in controlled experiments of Marshall in 1920, of Gamble in 1937, of Stewart and Rourke and of Schemm in 1942, of Wolf in 1945 and of others more recently. While the fact is certain that the forcing of fluids can, under proper circumstances, lead to dehydration, the efficacy of the procedure as a primary means of treating edema is not generally accepted.

The Dehydrating Effects of Water. Wolf observed that when normal subjects drink from 20 to 200 ml. of water every 10 min. for from 3 to 7 hr., urine flow increases rapidly and is sustained at a level some 8 per cent above intake. If one includes insensible water loss through lungs and skin in balance calculations, it is evident that total output of water can exceed intake by as much as 15 to 20 per cent. Wolf observed that urinary chloride concentration tended to be high at the start of the drinking period, to drop sharply over the first hour or so, and to reach a plateau of 1.2 mg. per ml. (33 mEq. per liter), after 3 hr. If urine flow were sustained for 7 hr. at the maximum rate of 20 ml. per min, the total salt lost would be equivalent to that contained in 2 liters of extracellular fluid, and at the end of diuresis, body weight would decrease 2 Kg. Two facts militate against such an ideal response in the edematous patient: diuresis is rarely as adequate as in the normal, in consequence of salt retention, the plateau of minimum urinary sodium and chloride concentration is lower. Furthermore, there is the ever present hazard of inducing acute dilutional hyponatremia of serious proportions.

The Schemm Regimen. Schemm has noted that while the mildly edematous patient does well on a fluid intake of 2500 to 3000 ml. per day, the severely edematous patient when first seen is occasionally seriously dehydrated in the sense that osmolality of the body fluids is increased in consequence of prolonged and ill-advised fluid restriction. For such patients Schemm recommends as much as 8 to 10,000 ml. of fluid for a day or so and 4 to 5,000 ml. per day thereafter. If such amounts of water cannot be tolerated by mouth, he recommends 500 to 1000 ml. of isotonic

fluids. However, many seriously ill patients cannot tolerate the dilution necessary to attain a significant saluretic response, if indeed it can be attained at all.

A point of some interest is the incapacity of the seriously ill edematous patient to exhibit water diuresis in response to hypotonic expansion of body fluids. Failure to respond to the ingestion of water with increased urine flow has been generally explained in terms of excessive secretion of ADH. The supraoptico-hypophyseal antidiuretic mechanism, although primarily sensitive to the osmotic pressure of the body fluids, has been considered to be subject to secondary control by the volume regulatory mechanism. This view has been questioned by Lamdin et al who have found that alcohol, which inhibits release of ADH from the neurohypophysis, does not restore water diuresis in patients with cirrhotic, nephrotic and cardiac edema. Recent experiments of del Greco, Kleeman, Berliner and their respective associates demonstrate that an acute reduction in glomerular filtration rate limits water diuresis. A sufficient reduction in filtration rate (20 to 30 per cent) causes oliguria of hypertonic urine, even though body fluids are dilute and secretion of ADH is suppressed. These facts suggest that reduction in filtration rate in the edematous patient may contribute to the reduced diuretic response to water loading. Other factors no doubt contribute. The author believes that excessive secretion of ADH may play some role in limiting water diuresis in edema, however, it cannot be the entire explanation; reduced filtration rate and other unknown factors also play significant roles.

SUMMARY

Providing the intake of sodium is sufficiently reduced, the intake of water has relatively little effect on the rate of accumulation of edema. In normal subjects and in some edematous patients, large water loads cause the development of negative sodium balance. Water expels water and body weight is reduced. However, the seriously ill edematous patient may respond adversely to large water loads by developing hyponatremia and water intoxication without exhibiting either diuresis or saluresis. The intake of mod-

By definition, the saluresis which follows hyperhydration results from glomerulotubular imbalance due to relative glomerular preponderance. No less than three factors may play some role in its induction, although their relative importance cannot be assessed at the moment. Bartter has shown that water loading in a pitressin treated subject reduces the rate of urinary excretion of aldosterone (see Chapter V), and presumably its rate of glandular secretion as well. In consequence of reduced circulating hormone, the rate of reabsorption of salt by the renal tubule is reduced; salt excretion is increased. Hyperhydration also increases glomerular filtration rate. In the studies of Leaf, filtration rate increased from 20 to 30 per cent. More salt containing isotonic fluid is delivered from the proximal tubules into loops of Henle, distal tubules and collecting ducts. If the reabsorptive capacities of these segments of the nephron remain relatively unchanged, the greater filtered load of salt would be less completely reabsorbed and excretion would increase. If in addition, reabsorptive capacity is reduced because of reduced secretion of aldosterone, excretion would increase still more. A final factor of unknown significance is velocity of flow of tubular urine along the collecting ducts. It is possible, though by no means proven, that absorption of the final traces of sodium is rendered less complete by high rates of flow in water diuresis.

Role of Hydration in the Therapy of Edema. It seems reasonable to assume that extracellular fluid volume is controlled by a receptor-hypothalamic integrator-neurohumoral effector system, which determines the balance between filtered load and tubular reabsorption of sodium (see Chapter V). It is a truism to state that this mechanism operates abnormally in edematous patients. Dehydration, though it may reduce somewhat the volume of interstitial fluid, stimulates intensely mechanisms of salt retention; diuretic therapy is rendered less effective. Provision of ample water, though it expands extracellular and cellular volume, i.e., increases edema, lessens the intensity of salt conservation; diuretic therapy is rendered more effective. Hyperhydration actually promotes salt loss in those patients in whom filtration rate can be sufficiently increased and aldosterone secretion sufficiently depressed by tolerable degrees of hypotonic expansion of volume of body

fluids. However, many seriously ill patients cannot tolerate the dilution necessary to attain a significant saluretic response, if indeed it can be attained at all.

A point of some interest is the incapacity of the seriously ill edematous patient to exhibit water diuresis in response to hypotonic expansion of body fluids. Failure to respond to the ingestion of water with increased urine flow has been generally explained in terms of excessive secretion of ADH. The supraoptico-hypophyseal antidiuretic mechanism, although primarily sensitive to the osmotic pressure of the body fluids, has been considered to be subject to secondary control by the volume regulatory mechanism. This view has been questioned by Lamdin et al who have found that alcohol, which inhibits release of ADH from the neurohypophysis, does not restore water diuresis in patients with cirrhotic, nephrotic and cardiac edema. Recent experiments of del Greco, Kleeman, Berliner and their respective associates demonstrate that an acute reduction in glomerular filtration rate limits water diuresis. A sufficient reduction in filtration rate (20 to 30 per cent) causes oliguria of hypertonic urine, even though body fluids are dilute and secretion of ADH is suppressed. These facts suggest that reduction in filtration rate in the edematous patient may contribute to the reduced diuretic response to water loading. Other factors no doubt contribute. The author believes that excessive secretion of ADH may play some role in limiting water diuresis in edema; however, it cannot be the entire explanation; reduced filtration rate and other unknown factors also play significant roles.

SUMMARY

Providing the intake of sodium is sufficiently reduced, the intake of water has relatively little effect on the rate of accumulation of edema. In normal subjects and in some edematous patients, large water loads cause the development of negative sodium balance. Water expels water and body weight is reduced. However, the seriously ill edematous patient may respond adversely to large water loads by developing hyponatremia and water intoxication without exhibiting either diuresis or saluresis. The intake of mod-

erate quantities of water, ad libitum in the alert and responsive patient, some 2000 to 3000 ml. in the depressed or comatose patient, supplies water needs, provides for an adequate urine volume and renders the patient more responsive to diuretic therapy. Restriction of fluid intake, except immediately after paracentesis or massive diuresis, is inhumane and without effect on the ultimate accumulation of edema, for salt retention continues and the body is diluted when water restriction is relaxed. Furthermore, water restriction reduces the patients response to diuretic therapy.

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Chapter XIV

OSMOTIC DIURESIS

In response to prolonged thirsting and fasting, the urine flow of a normal individual decreases to 0.1 to 0.3 ml. per min. and urine osmolality increases to a maximum of 1200 to 1400 mOsm. per liter, i.e., to a value some 4 to 5 times that of the plasma. Since urine osmolality is determined largely by content of urea and electrolytes, minimum urine volume is ultimately dependent on the load of these substances demanding excretion. Any increase in load must result in an increase in volume. When any excretory solute, whether a normal urinary constituent or a foreign substance, is administered in a concentration higher than that in which it can be eliminated, water is abstracted from the body. The solute serves as an osmotic diuretic. Were water alone to be abstracted from the body, no useful end would be achieved, for thirst would drive the individual to restore his water deficit. Such therapeutic benefits as derive from the use of osmotic diuretics result from the fact that sodium excretion increases more or less in proportion to the increase in urine flow.

Osmotic diuretics are not especially potent therapeutic agents and their limited clinical utility scarcely justifies an extended discussion. However, the experimental exploitation of osmotic diuretics has contributed greatly to an understanding of renal function. Furthermore, all effective diuretics in some degree induce osmotic diuresis, for by inhibiting the reabsorption of sodium and either chloride or bicarbonate ions, they increase the osmotic load of electrolytes demanding excretion. The excretion of electrolytes obligates the excretion of water, extracellular volume is reduced, and body weight declines. The reader will find that this chapter is more concerned with the functional than with the therapeutic implications of osmotic diuresis.

erate quantities of water, ad libitum in the alert and responsive patient, some 2000 to 3000 ml. in the depressed or comatose patient, supplies water needs, provides for an adequate urine volume and renders the patient more responsive to diuretic therapy. Restriction of fluid intake, except immediately after paracentesis or massive diuresis, is inhumane and without effect on the ultimate accumulation of edema, for salt retention continues and the body is diluted when water restriction is relaxed. Furthermore, water restriction reduces the patients response to diuretic therapy.

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papillary interstitium. The osmolality of the urine approaches that of the tissue forming the tip of the papilla. In osmotic diuresis, the presence of unreabsorbable solute in the glomerular filtrate restricts the isotonic reabsorption of sodium and water in the proximal tubules. Since distal reabsorption is limited, a much larger volume of isotonic fluid is delivered into the collecting ducts. One may theorize that the reabsorption of more water at this site reduces the hypertonicity of the papillary tissue, hence reduces the final osmolal concentration of the urine, and increases the volume flow of urine. The mechanisms of increased urine flow and increased sodium excretion in osmotic diuresis will be considered in the following pages.

Osmotic Load, Urine Osmolality, and Urine Flow are interrelated in the manner described in Figure 28 A, B, redrawn in idealized form from data of Raporort *et al.* Figure 28A describes the relationship between urinary osmotic load and urine flow in a series of young normal subjects fasting and thirsting for 16 hrs. prior to the experiment. Urine osmotic load is defined as the rate of excretion of osmotically active solutes, namely the product of urine osmolality and urine flow ($U_{osm} \times V$). In control observations of Figure 28A, both urine osmotic load and urine flow were low. The data grouped at the lower left hand end of the curve. The urine osmotic load was increased progressively by the intravenous infusion of a hypertonic solution of one of a variety of solutes. Urine flow increased to values as high as 22.8 ml. per min. The relationship between urine flow and urine osmotic load was the same, independent of the nature of the solute, and was thus dependent solely on rate of excretion of osmotically active particles, *not on their chemical constitution. The solutes included* glucose, urea, mannitol, sucrose, sorbose, sorbitol, xylose, creatinine, sodium sulfate, sodium para-aminohippurate and sodium chloride. Experimentally, any one of these solutes could be employed as an osmotic diuretic, practically, one would avoid sodium salts in the treatment of edema. Urea is the only one of these eleven solutes employed therapeutically.

Figure 28B describes the relationship between urine flow and plasma and urine osmolality in the same series of experiments from

Osmotic Work. The formation of urine hypertonic to plasma involves the performances of osmotic work. Osmotic work is some function of the product of the urine/ plasma osmolal concentration ratio, i.e., the degree to which the urine is osmotically concentrated with respect to the plasma, and the volume of urine elaborated per unit time, i.e., the urine flow. Hervey, Rapoport, Newburgh and others have calculated minimum renal osmotic work under a variety of conditions, as though concentration of the urine were carried out as a thermodynamically reversible process. The formation of small volumes of maximally concentrated urine involves the expenditure of relatively little energy, roughly 0.6 gm. cal, per min. per 1.73M^2 surface area. If osmotically active excretory solutes are administered in hypertonic solution in progressively increasing quantities, urine flow increases and osmolal concentration decreases. However, osmotic work, related to the product of the volume and osmolal U/P ratio, increases some 7 fold to a limiting value of 4 0 gm. cal. per min. per 1.73 M^2 surface area. Under such conditions of osmotic diuresis, less work is performed per ml. of urine, but more work is performed per min. in consequence of increased flow. Obviously, the maximum urine concentration of 1400 mOsm. per liter at minimum flows, as observed in simple dehydration, cannot be assigned to a limitation of osmotic work capacity of the kidneys per se; rather it must find its explanation in some absolute restriction of the capacity of the nephron to concentrate the urine relative to the plasma.

According to the thesis of Wirz, Hargitay and Kuhn developed in Chapter IV, the osmotic work involved in the production of urine hypertonic to plasma is performed in the loop of Henle by *the pumping of sodium from ascending limb into the interstitium.* An increasing gradient of osmolal concentration is developed along the loop from the corticomedullary junction to the tip of the papilla and involves both tubular contents and interstitial fluid (see Fig. 10.)

In simple dehydration, a small volume of isotonic fluid containing all of the excretory products is delivered from distal tubules into collecting ducts. As the tubular fluid flows along the collecting ducts, water is reabsorbed by osmosis into the hypertonic

papillary interstitium. The osmolality of the urine approaches that of the tissue forming the tip of the papilla. In osmotic diuresis, the presence of unreabsorbable solute in the glomerular filtrate restricts the isotonic reabsorption of sodium and water in the proximal tubules. Since distal reabsorption is limited, a much larger volume of isotonic fluid is delivered into the collecting ducts. One may theorize that the reabsorption of more water at this site reduces the hypertonicity of the papillary tissue, hence reduces the final osmolal concentration of the urine, and increases the volume flow of urine. The mechanisms of increased urine flow and increased sodium excretion in osmotic diuresis will be considered in the following pages.

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Figure 28B describes the relationship between urine flow and plasma and urine osmolality in the same series of experiments from

which Figure 28A was derived. Urine osmolality decreased with increasing urine flow to approach plasma osmolality as an asymptote. In the control periods prior to infusion of solute, plasma osmolality averaged 295 mOsm. and urine osmolality 1400 mOsm. per liter. As a result of infusion of solute, plasma osmolality rose to

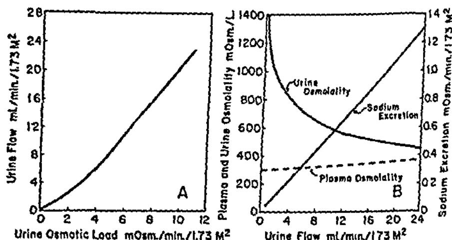


Fig. 28 Renal response to osmotic diuresis in man A. The relationship between urine flow and urine osmotic load. B. The relationship between urine osmolality, plasma osmolality, sodium excretion, and urine flow (Redrawn in somewhat idealized form from data of S. Rapoport, W.A. Brodsky, C.D. West, and B Mackler: *Am J Physiol*, *156*:433, 1949, and from data of S. Rapoport, C.D. West and W.A. Brodsky *Am. J. Physiol.*, *157*:363, 1949.)

365 mOsm. and urine osmolality decreased to 465 mOsm. per liter at the highest rate of urine flow, 22.8 ml. per min. Since the subjects were thirsting and hydropenic at the start, and since solutes were infused in highly hypertonic solution, the kidneys were maximally stimulated throughout the experiment by ADH to conserve water. As was pointed out above, ability to concentrate the urine is inversely related to the load of solutes demanding excretion. If solute load is small, high concentration is achieved; if solute load is large, the urine can be concentrated only to a limited extent and flow is correspondingly increased.

The Osmolal Clearance is defined as ml. per min. of plasma

completely cleared of osmotically active components and is calculated as follows:

$$C_{osm.} = \frac{U_{osm} \times V}{P_{osm.}}$$

where C_{osm} = osmolal clearance in ml. per min, U_{osm} = mOsm. per ml. of urine, P_{osm} = mOsm. per ml. of plasma, and V = ml. of urine per min. Osmolalities of plasma and urine are customarily measured cryoscopically, i.e., by freezing point depression, and thus include the contributions of both electrolytes and non-electrolytes. The osmolal clearance of the normal fasting individual varies between 2 and 3 ml. per min. at urine flows of 0.3 to 0.5 ml. per min, or more.

$$C_{osm.} = \frac{1.400 \text{ mOsm./ml.} \times 0.5 \text{ ml./min.}}{0.300 \text{ mOsm./ml.}} = 2.33 \text{ ml. per min.}$$

The osmolal clearance depends largely on the rate at which urea and electrolytes are cleared from the plasma and is, therefore, constant only within rather broad limits determined by the immediately preceding dietary history.

Osmolal Clearance and Water Conservation in Osmotic Diuresis. If the osmolal clearance is 2.33 ml per min. and the urine flow 0.5 ml. per min., as in the example cited above, the kidneys have in essence restored to the body 2.33 − 0.5 = 1.83 ml. per min. of pure water to cover extra-renal losses or to redilute hypertonic body fluids. Water conservation is defined as the difference between osmolal clearance and urine flow (C_{osm} − V.) It represents the volume of solute-free water which is reabsorbed from a volume of isotonic tubular fluid equivalent to the osmolal clearance, in order to increase its osmolality to that of the finished urine. Smith and his associates refer to water conservation as *negative free water clearance: negative*, because it represents reabsorbed water; *free water*, because it is solute-free. It is generally conceded that water conservation in the sense described above is effected in the collecting ducts.

The data presented in Table VIII are mean values derived from the curves of Figure 28 A, B. As urine osmotic load is increased from 0.7 mOsm. per min. (control) to 10 mOsm. per min. (osmotic diuresis), urine flow increases from 0.5 to 21.5 ml. per

min. and urine osmolality decreases from 1400 to 465 mOsm. per liter. Water conservation, i.e., the difference between osmolal clearance and urine flow, increases from 1.8 to 6 to 7 ml. per min., becoming roughly constant at urine osmotic loads greater than 3.0 mOsm. per min. Since water conservation in the collecting ducts is limited, the delivery of excessive volumes of isotonic fluid into these segments results in increased urine flow, i.e., osmotic diuresis.

Because of relative constancy of water conservation at high osmotic loads, Smith refers to it as T^c_m H_2O, the maximum capacity of the concentrating segment to reabsorb water. In terms of Wirz' hypothesis, this limited capacity of the tubules to conserve water can be explained in two ways. First, T^c_m H_2O might be related to a limited rate at which sodium can be pumped from ascending limbs of Henle's loops into interstitium and descending limbs. If the counter current multiplier and counter current exchange mechanisms described in Chapter IV were perfectly efficient (doubtful), the pumping of an amount of sodium equal to that contained in 6 to 7 ml. of isotonic proximal fluid from ascending to decending limbs of Henle's loops could account for the osmotic transfer of an equivalent volume of solute-free water across the collecting ducts. Second, T^c_m H_2O might represent a constant rate of osmotic transfer of water, limited by tubular permeability to water rather than by osmotic force. The data required to formulate a definitive explanation are not available.

Sodium and Water Excretion in Osmotic Diuresis. If, as pointed out above, diuresis were to cause only a loss of body water without loss of body sodium, it would serve no therapeutically useful purpose. Thirst would drive the patient to replace his water deficit and edema would reaccumulate. Actually, sodium is lost in osmotic diuresis in proportion to urine flow, a fact evident in Figure 28B. It is now generally accepted that increased sodium excretion in osmotic diuresis results from reduced reabsorption of this ion in the proximal tubules. Because less sodium is reabsorbed, less water is reabsorbed. More sodium and water are delivered into distal parts of nephrons. Because the reabsorptive capacities of these distal parts are limited, more salt and water are excreted.

TABLE VIII

RELATIONSHIPS OF OSMOLAL CLEARANCE, URINE FLOW AND WATER CONSERVATION IN OSMOTIC DIURESIS IN MAN

(Data are mean values taken from Figure 28)

Condition	*Urine Osmotic Load* $U_{osm} \times V$	*Urine Osmolality* U_{osm}	*Plasma Osmolality* P_{osm}	*Osmolal Clearance* C_{osm}.	*Urine Flow* V	*Water Conservation* C_{osm}-V
	(mOsm /min.)	*(mOsm /ml.)*	*(mOsm /ml.)*	*(ml./min.)*	*(ml /min.)*	*(ml./min.)*
Control	0.7	1.400	0.30	2.3	0.5	1.8
Osmotic Loading	2.0	0.625	0.30	6.7	3.2	3.5
Osmotic Loading	4.0	0.597	0.31	12.9	6.7	6.2
Osmotic Loading	6.0	0.545	0.33	18.2	11.0	7.2
Osmotic Loading	8.0	0.500	0.35	22.8	16.0	6.8
Osmotic Loading	10.0	0.465	0.36	27.8	21.5	6.3

An explanation of reduction of proximal tubular reabsorption of sodium and water in osmotic diuresis will be developed in terms of hypothetical data presented in Table IX. Under normal conditions, the glomerular filtrate is assumed to contain cations (exclusively sodium) in a concentration of 140 mOsm. per liter and anions (exclusively chloride and bicarbonate) in the same concentration. Glucose, amino acids and other valuable constituents are present in a concentration of 10 mOsm. per liter. Waste products are present in a concentration of 6 mOsm. per liter.[17] The sum of osmotically active components is 296 mOsm. per liter. The volume of filtrate is 100 ml. per min.

In the course of passage of the glomerular filtrate along the *proximal tubule, it is assumed, as in Chapter IV, that some* 7/8th of the sodium, chloride and bicarbonate and all of the valuable non-electrolyte constituents are reabsorbed. This provides the osmotic motive force to reabsorb 7/8th of the filtered water. *At the end of the proximal tubule, volume is reduced from* 100 ml. to 16.7 ml. per min., namely to 1/8th of its original value. By virtue of water reabsorption, excretory products are concentrated 8 times, from 6 to 48 mOsm. per liter. Much evidence indicates that the *proximal tubular fluid remains isosmotic with plasma; therefore,* total osmolal concentration remains unchanged at 296 mOsm. per liter. For this to be true, the concentrations of cations and anions must be slightly reduced, each to 129 mOsm. per liter. The rate of *delivery of cations and anions from the end of the proximal* segment into more distal parts of the nephron is 4.31 mOsm. per min. (129 mOsm./L + 129 mOsm./L) × 16.7 ml./min./1000. Under normal conditions, most of the 16.7 ml. of water and essentially all of the 4.31 mOsm. of electrolyte are reabsorbed each minute in the distal parts of the nephron.

In profound osmotic diuresis, the significant change in the composition of the plasma and glomerular filtrate is a marked increase in concentration of excretory products, in the example cited in the lower part of Table IX, to 100 mOsm. per liter. As a consequence,

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[17]Waste products such as urea and uric acid are in part reabsorbed in the proximal segment. The 6 mOsm per liter represents that moiety present in the filtrate which is not reabsorbed in the proximal tubules.

the sum of osmotically active components is increased to 390 mOsm. per liter. As the filtrate flows along the proximal tubule, all valuable nonelectrolyte components are reabsorbed. However, the reabsorption of cations, anions and water is reduced. According to Wesson and Anslow, the proximal reabsorption of sodium is retarded by the development of a critical gradient between tubular urine and plasma. This critical gradient develops because excretory products in the tubular urine limit the reabsorption of

TABLE IX

HYPOTHETICAL DATA ILLUSTRATING THE PROBABLE ORIGIN OF NATRIURESIS AND ENHANCED URINE FLOW IN OSMOTIC DIURESIS

	Glomerular Filtrate		*Fluid at End of Proximal Tubule*
		Normal	
Cations	140 mOsm /L		129 mOsm./L
Anions	140 mOsm./L		129 mOsm./L
Glucose, amino acid, etc.	10 mOsm./L		0 mOsm./L
Excretory products	6 mOsm./L	× 8 =	48 mOsm./L
ΣOsmotic components	296 mOsm./L		296 mOsm./L
Volume	100 ml./min	× ⅛ =	16 7 ml./min
ΣCations and anions at end of proximal tubule			4 31 mOsm./min.
		Osmotic Diuresis	
Cations	140 mOsm./L		80 mOsm /L
Anions	140 mOsm./L		80 mOsm./L
Glucose, amino acids, etc.	10 mOsm./L		0 mOsm./L
Excretory products	100 mOsm /L	× 2.3 =	230 mOsm./L
ΣOsmatic components	390 mOsm./L		390 mOsm./L
Volume	100 ml./min.	× 1/2.3 =	43 ml./min
ΣCations and anions at end of proximal tubule			6 88 mOsm /min.

An explanation of reduction of proximal tubular reabsorption of sodium and water in osmotic diuresis will be developed in terms of hypothetical data presented in Table IX. Under normal conditions, the glomerular filtrate is assumed to contain cations (exclusively sodium) in a concentration of 140 mOsm. per liter and anions (exclusively chloride and bicarbonate) in the same concentration. Glucose, amino acids and other valuable constituents are present in a concentration of 10 mOsm. per liter. Waste products are present in a concentration of 6 mOsm. per liter.[17] The sum of osmotically active components is 296 mOsm. per liter. The volume of filtrate is 100 ml. per min.

In the course of passage of the glomerular filtrate along the proximal tubule, it is assumed, as in Chapter IV, that some 7/8th of the sodium, chloride and bicarbonate and all of the valuable non-electrolyte constituents are reabsorbed. This provides the osmotic motive force to reabsorb 7/8th of the filtered water. At the end of the proximal tubule, volume is reduced from 100 ml. to 16.7 ml. per min., namely to 1/8th of its original value. By virtue of water reabsorption, excretory products are concentrated 8 times, from 6 to 48 mOsm. per liter. Much evidence indicates that the proximal tubular fluid remains isosmotic with plasma; therefore, total osmolal concentration remains unchanged at 296 mOsm. per liter. For this to be true, the concentrations of cations and anions must be slightly reduced, each to 129 mOsm. per liter. The rate of delivery of cations and anions from the end of the proximal segment into more distal parts of the nephron is 4.31 mOsm. per min. (129 mOsm./L + 129 mOsm./L) × 16.7 ml./min./1000. Under normal conditions, most of the 16.7 ml. of water and essentially all of the 4.31 mOsm. of electrolyte are reabsorbed each minute in the distal parts of the nephron.

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[17] Waste products such as urea and uric acid are in part reabsorbed in the proximal segment. The 6 mOsm. per liter represents that moiety present in the filtrate which is not reabsorbed in the proximal tubules.

sorption of sodium. The magnitude of that gradient has not been accurately defined in the mammalian kidney.

Characteristics of an Ideal Osmotic Diuretic include the following. (1) It should be restricted in its distribution in the body to the extracellular fluid compartment. If, like urea, it penetrates cells, large doses must be administered. Although urea is the most commonly used osmotic diuretic, it is by no means ideal in all respects. (2) It should not be metabolized in the body. (3) It must be freely filterable through glomerular capillaries and (4) should not be reabsorbed by the renal tubules. (5) It should be readily absorbed from the gut on oral administration. (6) When administered in effective doses, it should cause no gastro-intestinal or general systemic disturbances. No osmotic diuretic has all these characteristics, and as a class, they are little used today. Urea and potassium salts have been most extensively used clinically as osmotic diuretics because they are readily absorbed from the gut and rapidly excreted in the urine. Potassium salts will be discussed in another connection in Chapter XVIII.

Use of Urea as an Osmotic Diuretic. Friedrich in 1892 first employed urea as a diuretic in patients with congestive heart failure and cirrhosis. Although he obtained a favorable response to doses as small as 2 to 14 gm. per day, others have found it necessary to administer from 30 to 100 gm. per day to obtain significant diuresis. In most instances from 50 to 60 gm., divided into 3 doses and administered immediately after meals, produces as great a diuresis as is likely to be obtained. The increase in daily urine output may be 2-to 4-fold in patients in whom the prediuretic output is from 300 to 700 ml. The diuretic response is roughly comparable to that induced by xanthines.

Urea is rather extensively reabsorbed by the renal tubules, i.e., some 40 to 70 per cent of that filtered is returned to the blood stream. Most investigators believe that urea is passively reabsorbed, although Schmidt-Nielsen maintains that, in part, transport is active. Shannon has demonstrated that under normal conditions as much as 40 per cent of the filtered urea may be reabsorbed in the proximal tubules, a value reduced in profound osmotic diuresis to about 10 per cent. An additional 10 to 40 per cent is reabsorbed

water and thus dilute the sodium of the tubular fluid. Failure of sodium reabsorption leaves sodium in the tubular urine, which by its own osmotic effect still further prevents reabsorption of water. This concept is illustrated in the data of Table IX. If as a result of reabsorption of sodium, glucose and amino acids, volume is reduced to 43 ml. per min., excretory products are concentrated to 230 mOsm. per liter. To meet the requirement of isotonicity of proximal urine,[18] the concentration of cations and anions must be reduced to 80 mOsm. per liter. This represents a gradient of 80/140 mOsm. per liter between tubular fluid and plasma, sufficient to prevent further reabsorption of sodium. There would be delivered into more distal parts of the nephron 43 ml. of water containing 6.88 mOsm. per min. of cations and anions (80 mOsm./L. + 80 mOsm./L) × 43 ml./min./1000). If reabsorption of electrolyte and water by the distal nephron is limited to 4.31 mOsm. per min. and to some 16 ml. per min., respectively, urine flow would increase to more than 20 ml. per min. and electroylte excretion to 2.57 mOsm. per min. (6.88 — 4.31 = 2.57). The sodium moiety would be 1.28 mOsm. per min., a value of the proper order of magnitude (*cf.* Figure 28B.)

Mudge, Foulks and Gilman and more recently Thompson have claimed that no absolute gradient exists which stops sodium transfer in the proximal tubule. Rather rate of reabsorption of sodium is reduced both by a reduction in concentration of sodium and by a reduction in the length of time that the fluid is in contact with the tubular epithelium. Recently Windhager et al have shown, by perfusion experiments on the proximal tubule of Necturus, that an absolute limiting gradient exists. If the sodium concentration of the tubular perfusate is less than 2/3rds that of plasma, sodium and water enter the tubule; if more than 2/3rds, sodium and water are reabsorbed. The author, therefore, favors the explanation of Wesson and Anslow, namely that accumulation of osmotically active solute in the proximal tubule establishes a gradient between tubular fluid and plasma which prevents further reab-

[18]Both Wirz and Gottschalk have shown by micropuncture of mammalian proximal tubules that the tubular fluid is isotonic with plasma, not only under normal conditions, but also in osmotic diuresis produced by the infusion of mannitol.

sorption of sodium. The magnitude of that gradient has not been accurately defined in the mammalian kidney.

Characteristics of an Ideal Osmotic Diuretic include the following. (1) It should be restricted in its distribution in the body to the extracellular fluid compartment. If, like urea, it penetrates cells, large doses must be administered. Although urea is the most commonly used osmotic diuretic, it is by no means ideal in all respects. (2) It should not be metabolized in the body. (3) It must be freely filterable through glomerular capillaries and (4) should not be reabsorbed by the renal tubules. (5) It should be readily absorbed from the gut on oral administration. (6) When administered in effective doses, it should cause no gastro-intestinal or general systemic disturbances. No osmotic diuretic has all these characteristics, and as a class, they are little used today. Urea and potassium salts have been most extensively used clinically as osmotic diuretics because they are readily absorbed from the gut and rapidly excreted in the urine. Potassium salts will be discussed in another connection in Chapter XVIII.

Use of Urea as an Osmotic Diuretic. Friedrich in 1892 first employed urea as a diuretic in patients with congestive heart failure and cirrhosis. Although he obtained a favorable response to doses as small as 2 to 14 gm. per day, others have found it necessary to administer from 30 to 100 gm. per day to obtain significant diuresis. In most instances from 50 to 60 gm., divided into 3 doses and administered immediately after meals, produces as great a diuresis as is likely to be obtained. The increase in daily urine output may be 2-to 4-fold in patients in whom the prediuretic output is from 300 to 700 ml. The diuretic response is roughly comparable to that induced by xanthines.

Urea is rather extensively reabsorbed by the renal tubules, i.e., some 40 to 70 per cent of that filtered is returned to the blood stream. Most investigators believe that urea is passively reabsorbed, although Schmidt-Nielsen maintains that, in part, transport is active. Shannon has demonstrated that under normal conditions as much as 40 per cent of the filtered urea may be reabsorbed in the proximal tubules, a value reduced in profound osmotic diuresis to about 10 per cent. An additional 10 to 40 per cent is reabsorbed

in the distal tubules, the proportion varying as an inverse function of urine flow or directly with the degree of concentration of the urine. To whatever extent urea is reabsorbed, its efficacy as an osmotic diuretic is reduced. Because of relative non-toxicity and tolerance of high blood levels, tubular reabsorption does not ordinarily limit the capacity of the kidneys to excrete osmotically significant quantities of urea in the urine. If no diuresis occurs by virtue of severely reduced renal function (low glomerular filtration rate), the blood urea concentration may rise excessively.

The excretion of sodium and chloride is increased by the administration of urea more or less in proportion to the rate of excretion of urea. The excretion of excess water and electrolyte in urea diuresis as in other types of osmotic diuresis depends on diminished proximal tubular reabsorption as described above.

Two difficulties may be experienced in the use of osmotically adequate doses of urea. (1) In some patients, gastrointestinal disturbances, including nausea and vomiting, may preclude its use. If administered immediately after meals, little disturbance is usually encountered. All patients complain of its disagreeable taste, which can be only partially masked by flavoring agents. (2) In patients with severely reduced renal function, nitrogen retention occurs, accompanied by weakness, lassitude and loss of appetite, necessitating discontinuation of the drug. In patients with marked liver insufficiency, ammonia toxicity and hepatic coma may result as a consequence of conversion of urea to ammonia in the gut.

Except in patients with severe renal or hepatic insufficiency, urea is nontoxic, even on prolonged use. Undiminished diuretic potency over a period of years and a more or less additive response when combined with other agents increase its usefulness. It is properly claimed to be the safest, but certainly not the most effective of all diuretics.

Use of Other Solutes as Osmotic Diuretics. A variety of organic solutes, including glucose, sucrose, xylose, mannitol, sorbitol, sorbitan, and creatinine have been employed experimentally as osmotic diuretics. All must be administered intravenously to be effective; glucose, because of rapid metabolism; sucrose, because of digestion; and the remainder, because of poor intestinal absorp-

tion and resulting purgation. For this reason they are clinically less useful than urea and potassium salts.

The carbohydrates, glucose, sucrose, and xylose and the polyhydric alcohols, mannitol, sorbitol, and sorbitan are all effective diuretics when administered intravenously in hypertonic solution. Glucose is the only one of these substances significantly reabsorbed by the renal tubules or significantly metabolized in the body. Thus per gm. administered, it is the least effective, but its lack of toxicity and low cost largely counterbalance this objection. Sucrose, although not metabolized when given intravenously and only slightly reabsorbed by the renal tubules, may cause pathologic alterations in the kidney. Mannitol is inert, only slightly reabsorbed by the renal tubules and effective as a diuretic. Of the organic solutes it is the preferred. However, it must be given intravenously to be effective, and any patient sufficiently ill to justify intravenous therapy can be far more effectively treated in other ways.

Although intravenous sodium sulfate and sodium phosphate have been employed experimentally as osmotic diuretics, they have no place in the therapy of edema. Neither causes a net loss of sodium from the body. In fact both induce a positive sodium balance, for the anion is excreted in part with potassium, hydrogen and ammonia and a part of the sodium is retained in the body.

Limiting Factors in the Use of Osmotic Diuretics. For an osmotic diuretic to be effective, osmotically significant quantities of the agent employed must be excreted in the urine. If glomerular filtration rate is low, as it frequently is in edematous states, plasma concentration must be excessively high to deliver adequate quantities of the agent into the proximal urine. Since the capacity of an individual to tolerate a disturbance in osmotic pressure of the body fluids is limited, it may be impossible to obtain significant diuresis with osmotic agents. A second limitation of use is the well recognized fact that mechanisms for conservation of sodium are stimulated in edematous states. Even though greater than normal quantities of ions are delivered into distal portions of the nephron as a result of depression of proximal reabsorption, excessive reabsorption of sodium and chloride as ion pairs and excessive exchange of sodium for hydrogen,

potassium, and ammonia may limit excretion. Osmotic diuretics like all others are effective in the treatment of edema only insofar as they cause loss of body sodium.

SUMMARY

The normal human kidney can produce very small volumes (0.1 to 0.5 ml. per min.) of highly concentrated urine (1,400 mOsm. per liter) only when the load of osmotically active solutes demanding excretion is low (0.7 mOsm. per min. or less). If large quantities of solutes are excreted as a consequence of their oral or intravenous administration, urine flow increases and urine concentration decreases. If the urinary load of osmotically active solutes is as great as 10 mOsm. per min., urine flow increases to more than 20 ml. per min. and urine osmolality approaches that of the plasma. The excretion of sodium increases more or less in proportion to the increase in urine flow in osmotic diuresis. Increased excretion of sodium and water in osmotic diuresis is the result of decreased proximal tubular reabsorption. The reabsorption of sodium is limited by the inability of proximal tubular cells to pump this ion against a high concentration gradient. The magnitude of this limiting gradient is unknown; it may be of the order of 60 to 80 mEq. per liter. Such reabsorption of sodium and water as does occur concentrates the osmotic diuretic in the proximal tubular fluid. The osmotic effect which it exerts prevents further reabsorption of water. The water retained in the lumen dilutes the sodium and leads to the establishment of a critical gradient against which further transfer of sodium is impossible.

Osmotic diuretics are only moderately effective; hence are not widely employed clinically. Urea and potassium salts are the only osmotic agents which have been used to any extent in the treatment of edema.

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Chapter XV

XANTHINE AND AMINOURACIL DIURETICS

FOR nearly four decades during the latter part of the 19th and first part of the 20th centuries, xanthines were the mainstays of diuretic therapy. Caffeine was first used as a diuretic in a patient with congestive heart failure by Koshlakoff in 1864; theobromine was introduced by the pharmacologist von Schroeder in 1887; and theophylline was studied experimentally by Ach in 1900 and first employed clinically by Minkowsky and Doering in 1903. It was early recognized that theobromine and theophylline are superior to caffeine as diuretics; accordingly, the latter drug has been little used during the present century. In recent years, other more potent drugs have to a large extent replaced xanthines in the intensive diuretic therapy of grossly edematous patients. However, they are still useful in maintenance therapy, for they are adequately absorbed and reasonably well tolerated when given orally. They are most uniquely useful today in the potentiation of mercurial compounds in so-called "diuretic-fast" patients. In this role, aminophylline (theophylline ethylenediamine) excels.

The xanthines as a group exhibit a broad spectrum of pharmacological actions. They differ, however, in the degree to which they individually manifest these several properties. Caffeine is the most powerful of the three as a stimulant of the central nervous system and of skeletal muscle. It is least effective as a diuretic and as a stimulant of the cardiovascular system. Theophylline is the most potent diuretic and the most active stimulant of heart and circulation. It is also highly effective in relaxing the smooth muscle of the biliary tract and of the bronchial tree. It is intermediate in its actions as a central nervous and skeletal muscle stimulant. Theobromine, although less active as a diuretic per unit

weight of drug, can be tolerated in higher dosage than can theophylline, and acts for a longer period of time. Furthermore, it exhibits fewer undesirable side reactions and is, therefore, preferred as a diuretic by some. Recently in a search for improved orally effective compounds, the aminouracils have been observed to have diuretic activity and one, aminoisometridine (Rolicton) may well replace theobromine and theophylline for oral maintenance therapy of edema.

Chemical Constitution. Xanthine, as is evident in Figure 29, is a bi-cyclic compound, made up of a 6-membered pyrimidine ring and a 5-membered imidazole ring, the two rings sharing carbons 4 and 5 in common. Caffeine is 1, 3, 7-trimethyl xanthine; theobromine is 3, 7-dimethyl xanthine, and theophylline is 1, 3-dimethyl xanthine. Caffeine occurs naturally in coffee and tea; theobromine in cocoa, and theophylline in tea. The latter two compounds, however, are prepared synthetically, for theophylline, at least, occurs in nature in such small amounts that it is not com-

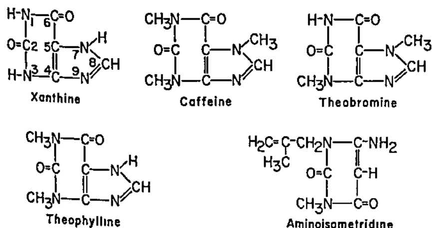


Fig 29. Structure of xanthine and pyrimidinedione diuretics.

mercially feasible to extract. The three compounds are weakly basic alkaloids and are sparingly soluble in water. They form soluble double salts or addition products with citric acid, with sodium glycinate, acetate, and benzoate, and with sodium and calcium salicylates. Theophylline also forms soluble salts with

ethylene diamine, (aminophylline), isopropanolamine, and choline. If the N-7 site of theophylline is substituted with the dihyroxypropyl group or with the diethylaminoethyl group, a water soluble neutral compound results. Aminoisometridine is 1-methallyl-3-methyl-6-amino pyrimidinedione. It is water soluble and administered as the parent compound. Insofar as the xanthines and the aminometridines contain the pyrimidinedione nucleus, they are structurally related.

Mechanism of Action of Xanthines. Mudge has recently stated that "the most remarkable thing about the xanthine diuretics is that so few facts have been clearly established concerning the mechanism of their action." This does not imply any early lack of investigative interest, for during the first few decades of this century, many papers, concerned with their mode of action, were published. This literature has been reviewed *in extenso* by Schmitz and by Vogl. However, the xanthines have been studied with the modern precise tools of renal physiology by relatively few investigators, no doubt because more effective diuretic agents were introduced at the same time that adequate methods of study were developed.

The early German workers, including Veil, Ellinger, Meyer, Ascher, Curtis and others maintained that the xanthines exert their diuretic effects peripherally by altering the binding of water to colloids or by increasing the delivery of chloride and other electrolytes from tissues to blood stream. Such peripheral actions have not been confirmed in recent studies employing more adequate experimental methods. Molitor and Pick ascribed the diuretic action of the xanthines to depression of the hypothalamic center which regulates water metabolism. In view of the fact that xanthines primarily increase the excretion of sodium and chloride and only secondarily affect water output, this view is untenable.

The observation of Schmidt and of Hartwich that caffeine produces diuresis in the isolated perfused frog kidney and that of Gremels and of Verney and Winton that it has a similar action in the Starling heart-lung-kidney preparation of the dog, definitely establishes a direct renal action, although of course it does not rule out other mechanisms as contributory. This view has been con-

firmed by Kupfer *et al.* who have observed that aminophylline causes diuresis in the pump-lung-kidney preparation of the dog perfused at constant pressure.

This direct renal action has been variously ascribed to (1) increased renal blood flow and glomerular filtration rate and (2) to depression of tubular reabsorption of salt and water. Phillips and Bradford first observed that caffeine causes the volume of the kidney to increase and inferred that diuresis results from renal vasodilation. Loewi *et al.* confirmed the observation and concurred in the interpretation. However Gottlieb, Brings, and Cushny as well, have pointed out that swelling of the kidney does not necessarily indicate vasodilation, instead it may reflect constriction of efferent vessels. Furthermore, diuresis occurs at times in the absence of change in kidney volume. Subsequently, more accurate measurements of renal blood flow in animals with the thermostromuhr and collection of venous outflow have shown that the xanthines do commonly cause increased renal blood flow. However, the increase in flow is often of relatively short duration and is certainly not a requisite of diuresis.

Richards and his coworkers have shown that caffeine increases the number of patent capillary loops in individual glomeruli and the total number of functioning glomeruli of the frog. Verney and Winton maintain that caffeine increases glomerular capillary filtering pressure and hence filtration rate by dilating afferent glomerular vesels to a greater degree than efferent. Blood flow may either increase, remain constant, or decrease depending on relative efferent tone, yet filtration pressure and filtration rate could, at least theoretically, increase under all circumstances.

Von Schroeder, who first studied the renal action of theobromine, attributed diuresis to stimulation of the secretory activities of renal tubular cells. Barcroft and Gremels supported this view with the observation that the drug increased renal oxygen consumption. However, others have found little evidence of increased renal metabolism, and net tubular secretion of water and salts, other than potassium, is not accepted today. Sobieranski in 1903 was the first to suggest depression of tubular reabsorption of salt and water, but his evidence can scarcely be accepted as significant,

even though subsequent work has amply confirmed his conclusion. Utilizing more or less adequate techniques for measuring glomerular filtration rate and for calculating filtered load of electrolyte, a number of investigators have shown in animals and in man that xanthine diuretics depress tubular reabsorption of sodium, chloride and water. Walker *et al.*, Davenport *et al.*, Blumgart *et al.*, and Crutchfield have emphasized depression of tubular reabsorption and have minimized the significance or denied the occurrence of changes in renal blood flow and filtration rate. However, in somewhat better controlled studies, Newman, Sinclair-Smith *et al.*, James *et al.*, Davis and Shock, and Weston and Escher have provided convincing evidence both of depression of tubular reabsorption and of increase in glomerular filtration. While diuresis may occur in the absence of an increase in filtration rate, it is definitely enhanced, if an increase occurs.

As was pointed out above, theophylline is the most potent cardiovascular stimulant of the xanthine group of drugs. When administered intravenously as aminophylline, cardiac output increases, central venous pressure drops, and oxygenation of blood improves. The drug, therefore, exerts some of the favorable effects of the cardiac glycosides. However, its actions although immediate are evanescent. It appeals to the author that insofar as aminophylline improves the circulatory dynamics of the patient in congestive failure, it will antagonize mechanisms of compensatory fluid retention. Thus it might be expected to increase glomerular filtration rate and reduce excessive secretion of aldosterone indirectly through extrarenal mechanisms responding to improved circulation. Presumably such effects would be mediated through the volume receptor-neurohormonal effector system and would be analogous to those induced by digitalis. Whether they are of significant magnitude is questionable.

Relatively little information is available concerning the mode of action of the aminouracils. Kattus et al have shown that 1-propyl-3-ethyl-6-aminouracil is an effective diuretic in patients with congestive failure, cirrhosis, and nephrosis. In the dog this drug blocks a modest fraction of the tubular reabsorption of sodium and chloride ions without exerting an appreciable effect on either

glomerular filtration rate or renal blood flow. It does not alter acid base balance and causes only a modest loss of potassium. The 1-allyl-3-ethyl analogue is equally effective and less irritating to the gastrointestinal tract. It was marketed briefly as aminometridine (Mictine), to be replaced by the 1-methallyl-3-methyl analogue, aminoisometridine (Rolicton), a compound equally potent, and accordingly to Clark and Hagans and to Settel, devoid of untoward side reactions.

No information is available concerning the nature of the enzyme systems blocked by either the xanthines or aminouracils, although it is perhaps reasonable to assume that they have a common mode of action. The site of action within the nephron is also unknown. Somewhat more surprising is the lack of information as to factors which modify the response to these agents, eg. acidosis, alkalosis, hyponatremia, hypochloremia etc.

Dosage and Route of Administration. Theobromine and theophylline may be administered by rectal suppository, by enema, orally, intramuscularly, and intravenously. Absorption from suppositories is notoriously unpredictable. Futhermore, continued use produces rectal irritation. Absorption of aminophylline by the lower bowel is adequate when the drug is instilled and retained following a cleansing enema.

The oral route is certainly to be preferred for maintenance therapy. However, gastric irritation, anorexia, nausea and vomiting frequently result when diuretically effective doses of the free alkaloids are administered. Theobromine on a weight for weight basis is much less irritating to the gastric mucosa than is theophylline. However, when the two drugs are compared in diuretically equivalent doses (theophylline is roughly 5 times as potent as theobromine), there is little reason to choose one in preference to the other. In general each drug is better tolerated when given in one of the many solubilized forms than when given as the free alkaloid.

Theobromine is usually administered as the sodium salicylate or calcium salicylate complex. Since the effective dosage is high, 3.0 to 5.0 gm. per day in divided doses, and since the sodium content of the sodium salicylate complex is appreciable, the calcium com-

pound is preferred. In general the compounds of theophylline are more widely used today than are those of theobromine because of their slightly greater activity. The free alkaloid, theophylline, in total daily dosage of 0.8 to 1.0 gm. (0.2 gm., 4 to 5 times a day), is probably the most effective oral form in which a xanthine diuretic can be given. However, such usage over any significent period of time is limited by high incidence and severity of gastric irritation. If given with colloidal aluminum hydroxide, gastric tolerance is increased. Less gastric irritation results when theophylline is administered as one of its soluble complexes. These complexes include theophylline sodium glycinate (0.3 gm., 3 to 4 times a day); dihydroxypropyl theophylline (0.2 gm., 3 to 4 times a day); and choline theophyllinate (0.2 gm., 3 to 4 times a day). Theophylline ethylenediamine (0.2 gm., 3 to 4 times a day) is somewhat less well tolerated orally than the above compounds, but irritation can be lessened by coadministration of aluminum hydroxide.

The xanthines are usually administered for periods of 4 consecutive days separated by drug-free intervals of 3 days. The rationale is presumably the following. It is claimed that habituation to xanthines destroys diuretic efficacy and that interrupted courses of treatment avoid this complication. More certain is the fact that gastrointestinal complaints increase in severity more or less in proportion to magnitude of dose and duration of therapy. It is, therefore, advisable to determine the smallest dose capable of maintaining dry weight and to devise some regimen which provides intermittant relief for the digestive tract.

Aminophylline, theophylline sodium glycinate, and certain other preparations are suitable for parenteral administration. In general the intramuscular route is preferred due to slower absorption, more prolonged action, and avoidance of the untoward consequences of too rapid intravenous injection. If the compounds are diluted and given very slowly, intravenous administration has the advantage of avoidance of the pain which follows intramuscular injection. However, too rapid intravenous injection causes giddiness, anxiety, palpitation, tachypnea and hyperpnea followed by nausea, vomiting, and syncope. A few deaths following rapid

intravenous administration have been reported. If the response to oral therapy is inadequate, it would seem wise to turn to more effective agents rather than to resort to intramuscular or intravenous administration of xanthines. An obvious exception to this rule is coadministration of intramuscular mercurial diuretics and either intramuscular or intravenous aminophylline in the treatment of the "diuretic-fast" patient, a procedure discussed subsequently in Chapter XVI in connection with mercurial diuretics.

Aminoisometridine is administered orally in a maintenance dose of 0.2 gm., 2 to 4 times a day. For short periods during the induction of diuresis, as much as 0.5 gm. may be given 3 to 4 times a day. The large dose may produce anorexia and nausea; the smaller maintenance doses produce few or no symptoms. Preliminary studies indicate that the drug is equally or more effective than the theophylline complexes and much better tolerated orally. Further experience will be necessary to assess the true value of aminoisometridine.

Toxicity. Signs and symptoms of gastrointestinal irritation from oral administration of xanthines and of systemic intoxication from rapid intravenous administration have been described above. Occasionally signs of excessive central nervous stimulation occur, although they usually constitute no problem with the common therapeutic doses. Theobromine produces less central nervous stimulation than theophylline.

Indications and Contraindications. Xanthines and aminoisometridine are on the whole relatively benign drugs, useful in oral maintenance therapy of edematous patients who do not exhibit overly intense salt and water retention. It is stated that the xanthines are most effective in the congestive failure of arteriosclerotic and hypertensive heart disease and less effective in rheumatic heart disease, cirrhosis with ascites, nephrosis and the nephrotic stage of chronic nephritis. They are frequently effective in the treatment of pre-eclampsia and premenstrual edema and tension. Aminoisometridine has not been used for a sufficient period to define its therapeutic limits, although it is probable that they are much the same as those of the xanthines. For several decades the xanthines were the only agents available for use in

severely ill patients intolerant to mercurial diuretics, and were given parenterally in such instances. Now there is a wider choice of drugs suitable for use in mercury intolerant patients and indications for parenteral therapy are less frequent.

Intolerance to xanthine diuretics is rare and manifestations of hypersensitivity are mild. The intravenous use of aminophylline is to be avoided in patients with recent extensive myocardial infarcts because the drug stimulates the myocardium intensely and may induce arrhythmias. The xanthines reduce clotting time and prothrombin time. It is possible that they might promote phlebothrombosis in severely ill or elderly patients, although there is no evidence that this is so. Xanthines depress the hepatic conversion of ammonia to urea in vitro. They probably should not be administered to patients with marked hepatic insufficiency because of the possibility of precipitating liver coma.

SUMMARY

Theophylline in one of its numerous solubilized forms is a useful drug for the oral maintenance therapy of edematous patients. Because it is somewhat more effective than theobromine, it is probably the drug of choice. The xanthines in general depress a modest fraction of the renal tubular reabsorption of sodium, chloride and water, increase the excretion of potassium slightly, have no effect on urine pH and ammonia excretion, and cause no significant disturbance in acid base balance. Theophylline frequently increases renal blood flow and glomerular filtration rate, especially in those patients without organic renal disease in whom these discrete functions are depressed. When filtration rate is increased, the diuretic response is enhanced. The aminouracils have similar renal actions except that they have little or no effect on glomerular filtration rate or renal blood flow.

Oral administration of theophylline in diuretically effective doses is frequently limited by gastrointestinal irritation. Use of the more benign solubilized forms or coadministration of colloidal aluminum hydroxide reduces irritation and permits more effective therapy. Aminoisometridine is largely devoid of irritating properties. Xanthines and aminouracils are most effective in those

patients who mildly retain salt and water. For more severely ill patients, requiring intensive parenteral therapy, other agents are more effective. In such patients, mercurial diuretics, potentiated by acidifying agents and by intramuscular or intravenous aminophylline, are especially effective.

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Chapter XVI

MERCURIAL DIURETICS

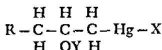
ORGANOMERCURIAL diuretics rank among the most valuable of the chemotherapeutic agents in use today. They have contributed immeasurably to the comfort, useful existence, and life span of countless patients over a period of nearly forty years. They are the time-proven standard of reference against which other diuretic agents are compared as to efficacy, reliability and toxicity. However, as is true of all potent drugs, certain risks attend the use of mercurial diuretics, risks which may be minimized by careful attention to such details of therapy as dose, route of administration, frequency of exhibition, and contraindications.

Mercury has an ancient, if not entirely venerable history as a diuretic. Paracelsus, early in the 16th Century, described the use of calomel as a purgative and diuretic. Various mixtures of mercury, calomel, digitalis, and squill were employed in the treatment of congestive heart failure as recently as the first decades of the present Century. However, mercurialism, with its attendant enteritis, stomatitis, renal damage, and occasional fatal outcome, discouraged any very intensive diuretic therapy with inorganic compounds of mercury.

Organomercurial compounds were first introduced as antisyphilitic agents, not as diuretics. In 1920, Saxl and Heilig described polyuria following each injection of Novasurol during the course of treatment of a patient for congenital syphilis. Subsequently, the diuretic efficacy of this drug was demonstrated in patients with congestive heart failure of rheumatic origin. Over the past 40 years a succession of organomercurial compounds have been produced, of increasing diuretic efficacy and diminishing toxicity. When carefully and conservatively used, the organomercurial compounds available today do not induce mercurialism.

However, fear of this eventuality has no doubt deprived many patients of the benefits of what as a class are the most effective of all diuretics.

Chemical Nature of Mercurial Diuretics. The mercurial diuretics in common use today as well as those undergoing clinical trial are substituted mercuripropyl compounds having the following basic structure.



The most significant feature of the diuretic structure is the terminal —C—Hg^{+} linkage which in neutral or alkaline solution is quite stable. The carbon mercury bond does not dissociate reversibly to give mercuric ion; if ruptured in acid solution, this bond does not spontaneously reform. The —Hg—X linkage, in contrast, is an ionic one; on dissociation, one mercury valence bond is freed to combine with tissue components. In 5 of the 7 drugs currently listed in New and Nonofficial Remedies, X is theophylline, in one, it is chloride; and in one, it is thioacetic acid. Theophylline, as the X substituent, increases solubility of the drug, decreases local irritation at the site of intramuscular deposition, and increases rate of absorption from that site. Whether it increases rate of urinary excretion and diuresis except by virtue of the fact that it increases absorption, is debatable, for the amount of theophylline contained in the usual therapeutic dose is relatively small. A striking decrease in cardiotoxicity and a further reduction in local irritation is observed when the X moiety is thioacetic acid as in mercaptomerin In chlormerodrin, X is chloride. This compound, while reasonably well absorbed from the gut, is irritating when given parenterally, accordingly, it is recommended for oral use only.

The OY substituent on the propyl chain is most commonly OCH_3 (methoxy). Its nature is of relatively minor importance in determining diuretic activity and either local or systemic toxicity. Its character is related to the solvent in which the mercuration reaction is carried out, and since the solvent most commonly employed is methyl alcohol, the methoxy substituent is the usual one.

The major structural differences among the several diuretics listed in Table X are evident in the R substituent. This grouping is allicyclic in mercurophylline and mercaptomerin; it is aromatic in mersalyl and merethoxylline; it is heterocyclic in mercumatilin; and it is acyclic in meralluride and chlormerodrin. In 6 of the 7

TABLE X

STRUCTURE OF THE COMMONLY USED ORGANO-MERCURIAL DIURETICS

ORGANOMERCURIAL COMPOUND	COMPLEXING AGENT	NAME
CH_3, CH_3, CH_3; NaO–C(=O)–(ring)–C(=O)–NH–CH_2–CH(O–CH_3)–CH_2–HgOH	Theophylline	*MERCUROPHYLLINE SODIUM* (Mercuzanthin)
	Sodium Thioacetate	MERCAPTOMERIN SODIUM (Thiomerin Sodium)
(benzene ring)–C(=O)–NH–CH_2–CH(OCH_3)–CH_2–HgOH; O–CH_2–C(=O)–O–Na	Theophylline	MERSALYL SODIUM AND THEOPHYLLINE (Salyrgan-Theophylline)
(benzene ring)–O–CH_2–C(=O)–O–Hg; C(=O)–NH–CH_2–CH–CH_2; O–CH_2–CH_2–OCH_3	Theophylline and Procaine	MERETHOXYLLINE PROCAINE (Dicurin Procaine)
CH_2–CH(OCH_3)–CH_2–HgOH; (coumarin ring) O, =O; C(=O)–ONa	Theophylline	MERCUMATILIN SODIUM (Cumertilin Sodium)
C(ONa)(=O)–CH_2–CH_2–C(=O)–NH–C(=O)–NH–CH_2–CH(OCH_3)–CH_2–HgOH	Theophylline	MERALLURIDE SODIUM (Mercuhydrin Sodium)
NH_2–C(=O)–NH_2–CH_2–CH(OCH_3)–CH_2–Hg–Cl	—	CHLORMERODRIN (Neohydrin)

compounds listed, the linkage between R and the propyl side chain is amide; in one it is carbon to carbon. At least one compound undergoing clinical trial has an ether linkage. R is by far the most important determinant of diuretic activity and toxicity. However, since the drugs accepted for clinical use have been selected from large numbers of compounds screened, it is not surprising that they are reasonably comparable so far as potency and toxicity are concerned. Furthermore, no pattern of R-structure has yet emerged which would enable one to design a compound of predictable properties. Practically any substituted allyl

compound ($R{-}CH_2{\cdot}CH{:}CH_2$) on mercuration in methyl alcohol will exhibit diuretic properties. Whether clinically useful must be determined emperically. It should, however, be pointed out that many, perhaps most, organic compounds of mercury are not diuretics. Subsequently, we shall consider two divergent views as to why certain compounds of mercury are diuretics, why others are not.

Renal Action of Diuretics. For some ten years after their introduction, organic mercurial diuretics were thought to exert their action by mobilizing salt and water in the tissues. Govaerts in 1928 observed that if one kidney of a dog is removed at the peak of a mercurial diuresis, and if its blood vessels are anastomosed with those of a non-diuretic animal, the transplanted kidney continues to exhibit polyuria. Bartram in 1932 noted that a minute dose of a mercurial diuretic, introduced into one renal artery, causes a prolonged increase in the excretion of urine by that kidney alone. A large dose, however, causes renal shutdown on the side injected and diuresis in the opposite kidney. These simple experiments demonstrated three fundamental facts concerning the action of mercurial diuretics: (1) they act directly on the kidneys; (2) they are fixed in renal tissue in the course of a single transit through the renal vascular bed and exert a prolonged diuretic effect; (3) in large dosage they are toxic and may cause renal shutdown. Such experiments do not exclude the possibility of peripheral action, but the observation of hemodilution prior to onset of diuresis, upon which this view was based, has not been confirmed in more recent studies.

Glomerular vs. Tubular Site of Action. A summary of a rather sophisticated modification of Bartram's experiment is presented in Figure 30. The two ureters of an anesthetized dog were separately catheterized and the animal was infused with creatinine to permit measurement of glomerular filtration rate. Following two control periods, shown at the left of the figure, a small dose of chlormerodrin labelled with radioactive Hg-203 was injected into the left renal artery. After a delay of 10 minutes, a brisk diuresis of sodium and water occurred which was restricted to the left kidney for some 60 to 75 minutes. Since diuresis developed without change

in filtration rate, it must have resulted from inhibition of tubular reabsorption of fluid and electrolyte. Uptake of the diuretic by the left kidney was incomplete; some obviously escaped into the general circulation, as evidenced by the appreciable concentrations of mercury in femoral arterial plasma. This accounts for the

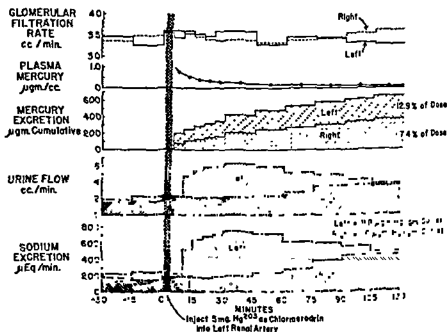


Fig 30. The diuretic response to the administration of 5 mg. of Hg^{203} as chlormerodrin into the left renal artery of a dog. (From R.F. Pitts: *Am. J. Med.*, *24*:745, 1958.)

accumulation of some of the diuretic in the right renal cortex, for its excretion in the urine formed by the right kidney, and for delayed diuresis which gradually developed on the right side. Evidence from a variety of sources indicates that mercurial diuresis results primarily from depression of tubular reabsorption of fluid and electrolyte, not from increase in filtered load.

Extent of Tubular Depression of Ion Reabsorption. In clinical practice the maximum dose of a mercurial diuretic, administered in a single injection, does not ordinarily exceed 2 ml. and contains not more than 85 mg. of mercury. For the average adult patient this represents slightly more than 1.0 mg. of mercury per Kg. of

body weight. Farah has observed that diuresis and natriuresis in dogs increase with dosage over the range of 0.5 to 5.0 mg. of mercury per Kg. Maximum excretion of sodium was obtained with doses between 3.0 and 5.0 mg. per Kg. and further increases to 25 mg. per Kg. did not enhance the response. While studies over such an extended range are out of the question in man, limited observations indicate that peak effects are obtained with 2.0 to 4.0 mg. of mercury per Kg.; thus man and dog exhibit roughly comparable sensitivities to the diuretic effects of these drugs. Since toxicity is related to dosage, even quantities of 2.0 to 4.0 mg. per Kg. cannot be justified in therapy.

The significant finding of these studies is that only a limited fraction of tubular reabsorption of sodium and chloride can be blocked by even the largest tolerated doses of drug. This fact is clearly illustrated in the data summarized in Table XI. In this experiment a dog was infused with isotonic saline at a rate of 10 ml. per min. for a period of 2½ hr. prior to, and throughout the course of the observations. Such extensive hydration, by expanding extracellular fluid volume, makes certain that diuresis will not be restricted by limited salt and water reserves. It also accounts for the high rate of urine flow and high rate of sodium excretion in the two initial control periods. During these periods, 93 per cent of the filtered sodium was reabsorbed, 7 per cent was excreted. A dose of 100 mg. of mercury as Mercuhydrin was then given intravenously. Urine flow increased from 9 to 17 ml. per min. and sodium reabsorption dropped from 93 to 80 per cent of that filtered, a decrease of 13 per cent. The dose of mercury was roughly 5.0 mg. per Kg. body weight, some 5 times the therapeutic dose in man, and sufficient to produce maximum depression of sodium reabsorption.

In other similar experiments maximum depression has varied between 12 and 20 per cent .Obviously, a large fraction, some 80 per cent or more of tubular reabsorption of sodium, is resistant to the action of mercurial diuretics. Blockade of only 20 per cent of sodium reabsorption might be related to depression of transport in a limited segment of the renal tubule, perhaps in the terminal part of the proximal segment. It might also be related to inhibition of

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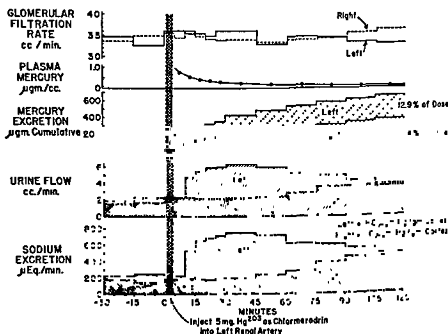


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an enzyme system which normally supplies a limited fraction of the energy for sodium transport throughout the proximal tubule. The author favors this latter view. Diuretic responses comparable to those shown in Table XI are not seen clinically, nor would they be desirable, for they would result in the precipitous discharge of excessive volumes of edema fluid, reduction in circulating blood volume, and circulatory collapse. At the end of this experiment 100 mg. of British Anti-Lewisite (BAL) was given intramuscularly. This substance complexes the diuretic and inhibits diuresis. Its mechanism of action will be considered later.

Effects on Water Reabsorption. It seems fairly certain that increased urine flow following mercurial diuretics is the osmotic consequence of inhibition of proximal tubular reabsorption of sodium and chloride ions (vide infra). It is not the consequence of direct interference with tubular mechanisms responsible for the formation of hypertonic urine, namely with ion pumps in the loops of Henle and with facultative control of the permeability of the distal tubules and collecting ducts to water. In other words mercurials do not block the mechanism for concentrating the urine, they merely render it less effective by increasing the osmotic load of salt. Thus Brodsky has observed identical relationships between urine flow and urinary osmotic load in hydropenic dogs infused with hypertonic mannitol and those given mercurial diuretics. Capps has noted in maximally hydrated normal subjects, exhibiting both water diuresis and mercurial diuresis, that infusion of antidiuretic hormone produces a decrease in urine flow and an increase in urine osmolality similar to that produced in control subjects exhibiting water diuresis alone. Thus the renal mechanisms which respond to endogenous and exogenous antidiuretic hormone seem basically unaffected by mercurial diuretics.

Blockade of Reabsorption of Sodium vs. Chloride. A fair amount of attention has been devoted to the questions: do mercurial diuretics primarily block chloride or sodium reabsorption? If as many claim, chloride reabsorption is specifically blocked, is increased sodium excretion merely a consequence of the necessity for eliminating in the urine equal numbers of cations and anions? Most would answer these questions in the affirmative for the

THE DIURETIC EFFECT OF A LARGE DOSE OF MERCUHYDRIN IN THE DOG AND THE ANTIDIURETIC EFFECT OF BAL

Urine Flow	*Glom. Filt. Rate*	*Plasma Sodium*	*Urine Sodium*	*Sodium* Filtered	*Sodium* Excreted	*Sodium* Reabsorbed	
(ml./min.)	*(ml./min.)*	*(mEq./L.)*	*(mEq./L.)*	*(mEq./min.)*	*(mEq./min.)*	*(mEq./min.)*	*(% filtered)*
10 ml. Saline per min. for preceding 2 hrs. and 30 min. intravenously							
8.93	80 0	151	90.5	11.49	0 81	10.68	93.0
9.07	81.7	152	89.7	11 81	0 81	11 00	93.1
100 mg. Hg as Mercuhydrin intravenously							
11.58	86.5	152	114	13.16	1.33	11.83	89.8
11 52	85.7	153	129	12.44	1.47	10.97	88.3
15 60	83.0	154	126	12.10	1 96	10.14	84 8
16 86	83.8	152	135	12.11	2.28	9 83	81.1
17 06	83.4	153	137	12.10	2.34	9.76	80.6
16.53	80 4	153	142	11.60	2.33	9.27	79.9
100 mg. BAL intramuscularly							
10.33	71.3	155	139	9 82	1.44	8.38	85.3
5.40	85.2	156	125	12.60	0.67	11.93	94 7
6.33	82.5	157	128	12.39	0 81	11 58	94.3

(From J. J. Duggan and R. F. Pitts: *J. Clin. Invest*, 29:365, 1950.)

both dog and man, are rapidly removed from the blood stream and concentrated in the kidneys; more specifically in the renal cortex. An experiment of Borghgraef is summarized in Table XII. Two dogs were given 1.0 mg. of mercury per Kg. intravenously in the form of Neohydrin. To facilitate analysis, the diuretic was synthesized with radiomercury Hg^{203}. Two hours later, at the peak of diuresis blood samples were drawn, the animals were sacrificed, and portions of representative tissues were removed. During the two hour period of diuresis, each of the two dogs excreted in the urine 40 per cent of the dose administered. The plasma concentrations of mercury were low, roughly 1.0 microgram (μgm.) per ml.

TABLE XII

COMPARISON OF THE DISTRIBUTION OF CHLORMERODRIN IN THE KIDNEY AND IN REPRESENTATIVE TISSUES OF THE DOG

	Dog A		*Dog B*	
Tissue	*μgm Hg/gm. or/ml.*	*Tissue/Plasma*	*μgm. Hg/gm. or/ml.*	*Tissue/Plasma*
Plasma	0.91	——	1.02	——
Kidney				
Cortex	163	179	131	128
Papilla	2.17	2.38	3.38	3.31
Liver	2.82	3.10	2.30	2.26
Spleen	1.93	2.12	0.86	0.84
Intestine	0.71	0.78	——	——
Adrenal	0.51	0.56	1.66	1.63
Heart	0.27	0.30	0.29	0.28
Muscle	0.16	0.18	0.11	0.11
Excretion in 2 hours	40.3% of dose		40.8% of dose	

(From R.R.M. Borghgraef and R. F. Pitts· *J. Clin. Invest.*, 35.31, 1956)

following reasons. Edematous patients undergoing mercurial diuresis commonly excrete more chloride than sodium in the urine, the sodium deficit being made up by potassium and ammonia. In the course of repeated diureses, loss of body chloride in excess of sodium results in hypochloremic alkalosis. If the plasma chloride drops below 90 to 95 mEq. per liter and if the plasma bicarbonate rises above 30 to 35 mEq. per liter, mercurial diuretics become ineffective. If normal chloride concentration is restored by the administration of ammonium chloride, responsiveness to mercurial diuretics returns. If hyperchloremic acidosis is induced, the diuretic activity of mercurials is markedly enhanced.

Strangely these observations do not prove primacy of blockade of chloride reabsorption. As Weston, Berliner and others have pointed out, they are compatible with primary blockade of sodium reabsorption in the proximal tubule. If less sodium is reabsorbed proximally, more sodium and hence more chloride will be delivered into the distal tubules and collecting ducts. If the mechanisms which exchange hydrogen, potassium and ammonia for sodium are stimulated, as they appear to be in edematous patients, less sodium than chloride will be excreted and the sodium deficit will be made up largely by hydrogen (more acid urine), potassium, and ammonia. If, as implied in Chapter IV, sodium transport is active and chloride transport passive, mercurial diuretics would of necessity block sodium rather than chloride reabsorption. This view is consistent with the finding of Giebisch that mercurial diuretics partially depolarize proximal tubular cells, i.e., lower transcellular potentials (see Chapter IV), presumably by slowing the rate at which the sodium pump ejects sodium from the cell into the peritublar fluid. However, there is no incontrovertible evidence in favor of this thesis. There may exist an independent active transport mechanism for chloride, and mercurials may block it. However, the author is inclined now, although not previously, to view sodium reabsorption as active, and in the proximal tubule, to be subject to partial blockade by mercurial diuretics.

Distribution of Mercurial Diuretics in the Body. It has been shown by Threefoot, Weston, Borghgraef and their respective associates, that mercurial diuretics, administered intravenously in

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tained. Kessler has shown that at least the last two of these postulates are not strictly true in the experimental animal. Thus diuresis wanes during the maintenance of the plateau of cortical concentration, no doubt limited in part by exhaustion of readily available extracellular fluid reserves. Perhaps the volume receptor system

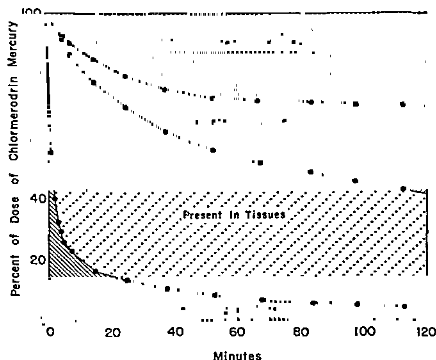


Fig. 31. The distribution in the body and urinary excretion of chlormerodrin by the dog as a function of time following the intravenous administration of 1.0 mg of Hg[203] per Kg. as diuretic drug. (From R.R.M. Borghgraef, R.H. Kessler and R.F. Pitts *J. Clin Invest.*, 35:1055, 1956)

operates to protect dwindling fluid reserves by reducing filtration rate and stimulating mercury resistant reabsorptive systems. Furthermore, Kessler has shown that certain non-diuretic organic compounds of mercury are concentrated in the renal cortex to a degree comparable to that to which the diuretics are concentrated. Apparently such simple explanations of onset, intensity and duration of mercurial diuresis as those proposed at the beginning of this paragraph are inadequate.

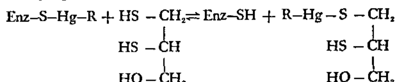
in the two experiments. In contrast, the concentrations of mercury in the renal cortex were high, 163 and 131 μgm. per gm. The specificity of binding by the cortex is illustrated by the fact that the concentrations of mercury in the renal papillae were only 2.2 and 3.4 μgm. per gm., considerably less than in the urine formed just prior to sacrifice. Tissue/plasma ratios indicate the degree of concentration in the several tissues relative to that in an equivalent amount of plasma. It is evident that only the renal cortex concentrates the drug appreciably.

Greif, using methods of homogenization and differential centrifugation, has shown that a part of the diuretic bound by the renal cortex is combined with mitochondria to form a stable non-dializable complex. However, most of the renal mercury is bound to soluble cytoplasmic proteins of cortical cells.

It is possible, by catheterizing the renal vein, by simultaneously collecting arterial blood, renal venous blood and urine, and by serially measuring renal blood flow, to describe the distribution in the body of a mercurial diuretic moment by moment after it is introduced into a peripheral vein. Such a description for a dog given 1 mg. of mercury per Kg. as chlormerodrin is summarized in Figure 31, taken from the work of Borghgraef. At zero time, i.e., immediately after introduction into a vein, the entire dose of the diuretic is contained in the plasma compartment, for little penetrates red cells. It immediately distributes into tissue; accordingly, plasma concentration falls rapidly during the first 20 mins. The kidney removes the drug from the peritubular blood, concentrates it within the renal cortex and secretes it into the urine. After 40 to 60 mins. the concentration in the renal cortex reaches a steady state: the diuretic is delivered from general tissue reservoirs to the kidney as rapidly as the kidney secretes it into the urine. Studies of Weston et al and Threefoot et al indicate that the distribution of Mercuhydrin in man follows a similar pattern and time course.

It is tempting to relate the onset of diuresis to the development of some critical concentration of diuretic in cortical tissue; the intensity of diuresis to the plateau concentration attained, and the duration of diuresis to the time some critical concentration is main-

integrity of sulfhydryl groups. This reaction is described in the following equation,



$$\text{Enz–S–Hg–R} + \begin{array}{l}\text{HS}-\text{CH}_2\\ \text{HS}-\text{CH}\\ \text{HO}-\text{CH}_2\end{array} \rightleftharpoons \text{Enz–SH} + \begin{array}{l}\text{R–Hg}-\text{S}-\text{CH}_2\\ \qquad\text{HS}-\text{CH}\\ \qquad\text{HO}-\text{CH}_2\end{array}$$

Inhibition of mercurial diuresis by BAL is illustrated in the last three clearance periods of the experiment presented earlier in Table XI.

Excretion of Mercurial Diuretics. Parenterally administered mercurial diuretics are eliminated largely by the kidneys, to a minor degree by the bowel, and to negligible extent in saliva, sweat and milk. Fecal excretion accounts for one-third or less of total excretion; the bulk of this moiety enters the gut in the bile. When mercurials are administered per os, a higher proportion is eliminated in the feces, for intestinal absorption of most of these drugs is relatively poor. Neohydrin and Cumertilin stand apart, in that they are better absorbed on oral administration.

Rate of urinary excretion of drug depends on route of administration, and is highest after intravenous injection, somewhat slower after intramuscular and subcutaneous injection and slowest after oral administration Combination of the drug with theophylline or thioacetic acid as the X substituent increases solubility, increases rate of absorption from the local site of deposition, and therefore increases rate of excretion. Estimates of the rate of excretion of the several accepted preparations by edematous patients range from 60 to 100 per cent of a single therapeutic dose in 24 hours. As much as 50 per cent may be excreted in 3 to 6 hours. Rarely is recovery of the administered dose complete, no doubt in fair part due to capricious chemical methods. It is certain that the vast majority of patients, given repeated injections, do not accumulate the drugs. However, it is equally certain that patients who do not give an adequate diuretic response, who are oliguric or anuric, or who have marked renal insufficiency and nitrogen retention do not excrete the drugs readily and may show cumulative toxicity if

Enzyme Inhibition in Diuresis. One of the most characteristic reactions of inorganic salts of mercury and of those organic compounds with one free mercury valence is the formation of mercaptides with thiols. It is a reasonable assumption to assign the diuretic properties of mercurial compounds to their ability to inhibit renal sulfhydryl enzymes by forming inactive mercaptide complexes. Wachstein and Meisel, Rennels and Ruskin, and others have noted that the activity of succinic dehydrogenase, demonstrable histochemically in the kidney of the rat by the neotetrazoleum reaction, is inhibited by the prior administration of mercurial diuretics in extremely high dosage (10 to 30 mg. of mercury per Kg. body weight). The inhibition is most pronounced in the third or straight descending segment of the proximal tubule. Unfortunately, doses equivalent to therapeutic doses in man have no demonstrable effect on succinic dehydrogenase activity. Therefore, inhibition of the enzyme may have more pathological than functional significance. Cafruny, *et al.*, using quantitative methods for histochemical identification of protein bound sulfhydryl groups in sections of kidney, have observed reductions in concentration in proximal tubules, loops of Henle, and collecting ducts following the administration of mercurial diuretics in amounts within the therapeutic range. It is possible that among these protein bound sulfhydryl compounds are enzymes which supply energy to the machinery which transports sodium ions.

The following equation, Enz-SH + $^{+}$Hg-R $\rightleftarrows$ Enz-S-Hg-R, implies that a mercurial diuretic ($^{+}$Hg-R) reacts reversibly with sulfhydryl enzymes, to yield inactive complexes. These enzymes have a greater affinity for diuretics than do monothiols such as cysteine and glutathione, but a lesser affinity than do dithiols such as BAL. This statement derives from the observation of Earle, Farah and others that mercurial compounds complexed with monothiols retain their diuretic properties; those complexed with dithiols do not. Dithiopropanol, BAL, administered at the peak of a mercurial diuresis, promptly reduces salt excretion and urine flow to the control range by complexing the diuretic and restoring the

dryl compounds. The same statement applies to the free valence of the mercury linked to the terminal carbon of the parent molecule; it too must be largely bound by thiols. The thesis that rupture of the carbon-mercury bond is a requisite of diuretic activity has recently been revived by Mudge and his colleagues. Mudge has observed a relationship between *in vivo* diuretic activity and *in vitro* acid lability in a series of organomercurial compounds. He explains the well recognized potentiation of mercurial diuresis by ammonium chloride in terms of the intracellular as well as extracellular acidosis which it induces. The greater the renal intracellular acidosis, the more the parent compound is broken down to diuretic mercuric ion. Inhibition of diuresis in metabolic alkalosis is presumed to result from greater stability of the parent compound. In view of Müller and Weiner's results, namely that most of the drug excreted in the urine is in the form of parent compound complexed with cysteine or acetyl cysteine, it is necessary to make the following assumption. Only a minute fraction of the drug taken up by the kidney, concentrated in the cortex, and secreted in the urine is diuretically active. The bulk of the diuretic serves no pharmacologically useful purpose; only the minute fraction which is broken down is active. In support of their thesis, Mudge et al have shown that the diuretic efficacy of Mercuhydrin (an acid-labile compound) is markedly affected by alterations in acid base balance; the efficacy of mercuric cysteine (an ionizable compound) is not. Furthermore, per mg. of mercury administered, the diuretic activity of mercuric cysteine[13] is considerably greater than that of Mercuhydrin, for all of its mercury is potentially available as mercuric ion.

While this thesis has its attractive aspects and may well be true, certain facts argue against it at the moment. The infusion of acetazoleamide and of potassium chloride, procedures which not only alkalinize the urine but presumably elevate the pH of the contents of renal tubular cells as well, do not alter the diuretic response

[13]This should not be construed as indicating that mercuric cysteine is a clinically useful diuretic; it is not, for it is highly toxic.

doses are repeated at short intervals in an attempt to force a response.

Mercurial diuretics are eliminated in the urine almost entirely by a process of active tubular secretion. Several lines of evidence point to this conclusion. Diuretics circulating in the blood stream are highly bound to the SH groups of plasma albumin. To the extent that they are bound, they are non-filterable through glomerular capillaries. The albumin-diuretic complex must however dissociate to some extent, for during passage of blood through peritubular capillaries, the drug is transferred to tubular cells. Tubular cells must therefore have a greater affinity for mercurial diuretics than do plasma proteins. Muller and Weiner have shown that mercurial diuretics are eliminated in the urine as complexes of the parent compound with cysteine or acetyl cysteine. Obviously, energy must be expended in breaking the strong linkage between diuretic and tubular cell in order to complex it with a monothiol to which it is less firmly bound, and transfer it into the urine. Calculations of Borghgraef, from the arterio-venous extraction studies mentioned previously, indicate that not less than 90 per cent and probably more nearly 100 per cent of urinary diuretic is eliminated by a process of active tubular secretion. It seems certain that a variety of organic compounds of mercury, having widely different structures, and inorganic mercuric ions as well are secreted into the urine as monothiol complexes by a common tubular mechanism. It is, therefore, likely that the carrier system combines with the free $—Hg^{+}$ valence common to all. Perhaps the transport mechanism is a basic one devised to rid the body of the traces of heavy metals which are contained in ingested food and water. The older concept that both excretion and diuretic activity can be explained in terms of glomerular filtration and partial tubular reabsorption of the mercurial compounds is patently incorrect.

The Mechanism of Enzyme Inhibition. Sollman some years ago postulated that organic compounds of mercury are diuretics by virtue of the fact that they decompose in the body to liberate mercuric ions. Of course mercuric ions exists in the body in vanishingly low concentration because of the ubiquity of sulfhy-

yl compounds. The same statement applies to the free valence the mercury linked to the terminal carbon of the parent olecule; it too must be largely bound by thiols. The thesis that pture of the carbon-mercury bond is a requisite of diuretic tivity has recently been revived by Mudge and his colleagues. ludge has observed a relationship between *in vivo* diuretic activity id *in vitro* acid lability in a series of organomercurial compounds. e explains the well recognized potentiation of mercurial diuresis y ammonium chloride in terms of the intracellular as well as tracellular acidosis which it induces. The greater the renal intra-llular acidosis, the more the parent compound is broken down diuretic mercuric ion. Inhibition of diuresis in metabolic alka-sis is presumed to result from greater stability of the parent ompound. In view of Müller and Weiner's results, namely that iost of the drug excreted in the urine is in the form of parent ompound complexed with cysteine or acetyl cysteine, it is neces-iry to make the following assumption. Only a minute fraction of ie drug taken up by the kidney, concentrated in the cortex, and ccreted in the urine is diuretically active. The bulk of the diuretic erves no pharmacologically useful purpose; only the minute raction which is broken down is active. In support of their hesis, Mudge et al have shown that the diuretic efficacy of lercuhydrin (an acid-labile compound) is markedly affected by lterations in acid base balance; the efficacy of mercuric cysteine an ionizable compound) is not. Furthermore, per mg. of mercury dministered, the diuretic activity of mercuric cysteine[19] is con-iderably greater than that of Mercuhydrin, for all of its mercury s potentially available as mercuric ion.

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[19]This should not be construed as indicating that mercuric cysteine is a clinically iseful diuretic, it is not, for it is highly toxic.

of the dog to Salyrgan or Neohydrin.[20] Furthermore, the induction of a marked extracellular and intracellular acidosis by the inhalation of 12 per cent CO_2 does not potentiate the action of mercurial diuretics, whereas a mild acidosis, induced by ammonium chloride, does. Finally, if inhibition of sulfhydryl enzymes is of significance in mercurial diuresis, it is not immediately apparent why compounds of the character of R—Hg^+ could not block them as well in vivo as in vitro. Mudge, however, maintains that divalent mercury is necessary to block two adjacent active sites on renal enzymes, one a sulfhydryl group, the other an amino or carboxyl group. This concept is illustrated to the left of Figure 32.

A somewhat different view has been outlined by Kessler and his colleagues, who suggested from their studies of a limited series of organomercurial compounds that steric configuration might be

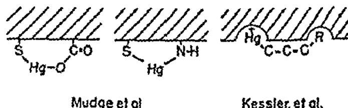


Fig. 32. Mechanism of enzyme inhibition by mercurial diuretics. Left, view of Mudge et al that the organic compound must be split to liberate divalent mercuric ion which combines with adjacent sulfhydryl and either carboxyl or amino groups. Right, view of Kessler et al that structural configuration of the organomercurial compound is significant. (From R.F. Pitts' *Am. J. Med.*, 24:745, 1958.)

of greater significance in determing diuretic activity. They proposed that for the compound to form a stable complex with renal enzymes, a terminal atom of mercury must be separated by three carbons or by an equivalent inter-atomic distance from a hydrophilic group. This concept is illustrated to the right of Figure 32. Although they recognized certain exceptions in Novasurol and Cumertilin, they proposed the thesis as a working hypothesis. It should be pointed out that mercuric cysteine has a structure similar

*In man, the administration of acetazoleamide depresses mercurial diuresis, the administration of potassium chloride does not.

to that proposed by Kessler, if it be permissible to substitute a sulfur atom for one of the carbons of the three unit chain. It is entirely possible that inorganic mercuric ions, administered in any form, ultimately reach the kidney as mercuric cysteine. Mercuric chloride, a reasonably effective though toxic diuretic, might actually be concentrated within the kidney as mercuric cysteine, and thus fulfill the structural requisites proposed. To avoid belaboring the point, it seems best to reserve for the future a decision as to whether the parent molecule is active per se or whether splitting of the carbon mercury bond is necessary for the development of activity.

Site of Diuretic Action and Site of Secretion of Organomercurial Compounds. The portion of the nephron within which organomercurial compounds exert their diuretic effects has long been a subject of controversy. One approach has been the study of the pathological lesions which result from the administratoin of both inorganic and organic compounds of mercury. When minimal necrotizing doses of inorganic compounds are given, the proximal tubule and more specifically its distal portion, shows evidence of pathological change. It was pointed out earlier that succinic dehydrogenase is inhibited in this same part of the tubule by relatively massive doses of mercurial diuretics. These two observations have been cited as evidence that mercurials block reabsorption in the terminal part of the proximal tubule. However, it is well to remember that an alteration in cell structure produced by a toxic dose of a compound need not necessarily indicate the site at which a specific functional change is induced by a therapeutic dose.

In man it has been shown that mercurial diuretics in therapeutic doses reduce the capacity of the renal tubules to reabsorb glucose and to secrete para-aminohippurate. Since these functions are presumed to be localized in the proximal segment of the renal tubule, the conclusion has been drawn that mercurial diuretics block salt reabsorption in the same segment. Unfortunately for this thesis, neither the reabsorption of glucose nor the secretion of para-aminohippurate by the kidney of the dog is significantly depressed by mercurial diuretics. Mercurial diuretics do not inter-

fere with acidification of the urine or with ammonia secretion. Since both the functions are presumed to reside in the distal tubules, it has been inferred that mercurial diuretics could not exert their characteristic effects at this site. The dangers of such derived arguments are evident if one considers the fact that mercurial diuretics partially inhibit the secretion of potassium, a function no less sure in its distal localization than ammonia and acid secretion. Clearance observations have been variously interpreted as indicating proximal blockade or distal blockade of salt reabsorption. the nature of the argument depending more on the bias of the investigator than on the validity of the reasoning.

Recent studies of Kessler et al and Vander et al, utilizing the "stop-flow" method for localizing functions in the nephron of the dog, have provided objective evidence that mercurial diuretics are excreted by, and exert their major inhibitory effects on ion reabsorption in the proximal segment. Figure 33 is a summary of data illustrating these points. The "stop-flow" method, originally described by Malvin, Sullivan and Wilde, is briefly the following. One ureter of a dog is catheterized through a small flank incission and an osmotic diuresis is initiated by the infusion of 20 per cent mannitol containing creatinine and para-aminohippurate. When the urine flow from the one kidney attains a value of 8 to 10 ml. per min., the ureteral catheter is clamped for a period of 6 to 8 min. One minute before the clamp is released, a gram of inulin or ferrocyanide is given intravenously. The clamp is released and over the succeeding 3 minutes some 30 to 40 samples of roughly 1.0 ml. are collected in rapid succession.

The reasoning behind this technique is the following. On clamping the ureteral catheter, pressure builds up rapidly within the tubular system; filtration ceases or at least slows markedly. An essentially stationary column of fluid is held in contact with the tubular epithelium for 6 to 8 min. During this prolonged period of contact, the tubular epithelium performs in exaggerated fashion those operations on the static column of fluid which it normally performs in lesser degree on the moving column. When the clamp is released, urine under pressure is ejected forcibly, the first samples coming from pelvis and more distal parts of the nephron,

later samples from more proximal parts. The final samples contain increasing amounts of ferrocyanide or inulin, substances which serve to mark the time of appearance and to quantify the admixture of fresh formed glomerular filtrate. Three blood and urine samples collected immediately before and three more collected immediately after the period of clamping serve to control the procedure.

Two experiments are summarized in Figure 33: one, a control; the other, performed during a diuresis induced by the intravenous injection of 1.0 mg. Hg^{203} per Kg. body weight as chlormerodrin. Plasma, control urine and the fractional urine samples were analyzed for creatinine, para-aminohippurate, sodium and, in the experiment in which chlormerodrin was given, for radiomercury as well. The urine/plasma concentration ratio for creatinine (U/P_{Cr}) shown at the bottom of the figure provides an indication of the site and degree of water reabsorption. U/P_{Cr} is highest in the distal part of the nephron, therefore water has been reabsorbed to the greatest extent in this region.

The U/P ratios for radiomercury, for sodium and for para-aminohippurate have been divided by the simultaneous U/P ratio for creatinine. This arithmetic device accomplishes two ends. First, it corrects for variable water reabsorption in the several parts of the nephron. Second, such ratios of U/P ratios have the connotation of clearances of the substance in question divided by filtration rate: if the numerical value is less than 1.0, the substance is reabsorbed;[21] if greater than 1.0, it is secreted.

Mercury is obviously secreted most avidly in the proximal tubule; i e., the $U/P_{Hg}/U/P_{Cr}$ attains a value of 3.0 in this segment. Depression of sodium reabsorption is also most evident in the proximal tubule. Thus the $U/P_{Na}/U/P_{Cr}$ in this segment is 0.15 in the control experiment; following the diuretic, it is 0.30. The distal tubular reabsorptive mechanism which is capable of reducing urinary sodium nearly to zero seems relatively little affected by the

[21]A ratio less than 1.0 indicates that the substance is reabsorbed, providing it is freely filterable through the glomerular capillaries. However, if the substance is highly bound to plasma proteins as are mercurial diuretics, a ratio less than 1 0 is not necessarily indicative of reabsorption.

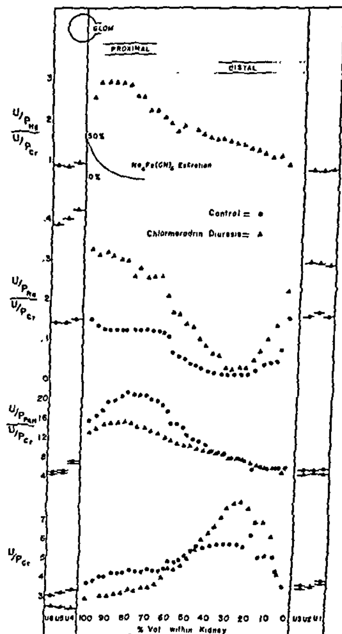


Fig. 33. "Stop-flow" experiments on the dog which localize inhibition of reabsorption of sodium by chlormerodrin and active tubular secretion of the diuretic drug to the proximal portion of the nephron. (From R.H. Kessler, K. Hierholzer, R.S. Gurd, and R.F. Pitts. *Am. J. Physiol.*, 194 540, 1958.)

action of mercury. Proximal secretion of para-aminohippurate is only moderately depressed, a fact which illustrates the specificity of action of mercurials on reabsorptive transport of ions. Certainly the major action of mercurial diuretics is to reduce proximal reabsorption of sodium and chloride. Any effects which it may exert on the distal tubule are minor.

DOSE AND ROUTE OF ADMINISTRATION

Mercurial diuretics have been administered to patients by all of the several possible routes: rectal, oral, intraperitoneal, subcutaneous, intramuscular, and intravenous.

The Intraperitoneal Route has been found unsatisfactory, causing undue irritation and yielding a poor diuretic response.

Administration by Rectal Suppository is occasionally effective, although the diuretic response is variable, delayed, prolonged, and generally unpredictable. At best, absorption through the rectal mucosa is poor and the dose must be correspondingly large. The most commonly used drug is Mercuhydrin, marketed in suppositories containing 190 mg. of mercury. The dose is one suppository per day inserted at bed time. Efficacy can be somewhat enhanced if an enema is given prior to insertion of the suppository. Mercuhydrin produces less rectal irritation than Salyrgan; the latter has produced ulceration. While rectal administration permits self medication, a goal worthy of achievement, the use of oral preparations is more likely to result in adequate diuresis.

Oral Administration. Two preparations are especially recommended for oral use, Neohydrin and Cumertilin. Both are somewhat better absorbed by the gut than are the other diuretics. Tablets of Neohydrin contain 10 mg. of mercury; tablets of Cumertilin contain 20 mg. The dose in either instance is 1 to 4 or more tablets per day, repeated daily or in interrupted courses. Some have used Neohydrin in dosage as high as 12 tablets per day for short periods. Oral use is frequently limited by epigastric discomfort, nausea, vomiting and diarrhea. Since these difficulties are more common with larger doses, therapy should start with two tablets per day, increasing as indicated to a maximum of six to eight. If eight tablets per day are insufficient, the oral route should

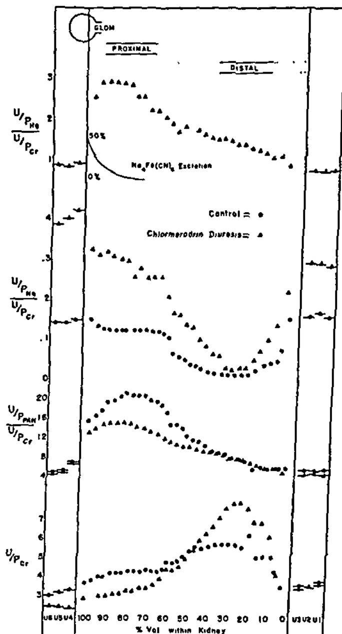


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Thiomerin. In spite of the fact that theophylline reduces tissue reaction, intramuscular injection of most mercurial diuretics causes some burning sensation and local tenderness. Dicurin contains 45 mg. of procaine base in each ml. to minimize local discomfort. Although the local anesthetic relieves pain, it does not alter tissue reaction; hence Dicurin should not be used subcutaneously. Thiomerin and Dicurin cause least local distress; Mercuhydrin is next best tolerated. Despite these drawbacks, the intramuscular route is commonly employed. Absorption is prompt, the overall diuretic response is equal to or greater than that following intravenous injection, there is no danger of thrombosis and slough from accidental infiltration of a vein, and the acute cardiac complications of intravenous administration, rare though they may be, are avoided. *There is no circumstance which justifies intravenous administration of mercurial diuretics, even though the practice is relatively common.*

Time Relations in Diuresis. The time of onset and the duration of diuresis vary with the route of administration; onset is delayed and duration is longer with oral and rectal administration than with parenteral. Following 1 ml. of Mercuhydrin intramuscularly, diuresis begins within 2 hr., reaches a maximum in 4 to 6 hr. and lasts for 12 to 24 hr. It is therefore advantageous to administer the diuretic in the morning so as to avoid disturbing the patients rest at night. In young individuals the diuresis is apt to be brisk, whereas in the elderly and weak, it may be less intense and more prolonged. The loss of as much as 14 liters of edema fluid in response to a single injection has been described. This is not an especially desirable therapeutic goal, for rapid loss of fluid increases the incidence of complications. Loss of 2 to 3 lb. per day is adequate, 5 lb., the maximum sought. In normal dogs and man, the intravenous administration of mercurial diuretics initiates a diuresis in ½ hr., which reaches a peak in the second hour, and is completed in about 6 hr. It is an interesting but unexplained fact that the onset of diuresis is delayed 10 min. following the injection of a minute amount of a mercurial diuretic directly into one renal artery of a dog.

probably be abandoned, because of the high incidence of gastrointestinal symptoms with large doses. In practice, oral mercurial diuretics are frequently ineffective; they rarely produce an adequate diuretic response in the severely ill patients; and in those patients in whom they are effective, other newer oral diuretics are equally active and often better tolerated.

Parenteral Administration. Of the three parenteral routes, intravenous, subcutaneous and intramuscular, the latter is the preferred. The accepted diuretics listed in Table X, all contain from 38 to 43 mg. of mercury per ml. of solution for injection. The range of therapeutic dosage in the adult is 0.5 to 2.0 ml. per day repeated as needed, i.e., weekly, semiweekly, or daily. The dose should be individualized for each patient in the same sense that the dose of digitalis is individualized. The patient in mild congestive failure should receive an initial dose of only 0.5 ml., the patient in severe failure, 1 to 2 ml., and the amount to be given subsequently should be adjusted in the light of the response to the initial dose. The smallest dose giving an adequate response (loss of 2 to 5 lb. per day) should be repeated at frequent intervals until edema fluid is fully discharged.

For children the dose should be proportionally smaller and *should not exceed 1.0 mg. of mercury per Kg. per day.* Several fatal reactions have been described in children, and in each instance the dose has been excessive.

De Graff *has shown that the combination of the* organomercurial component with equimolar quantities of theophylline greatly increases rate of absorption from the site of intramuscular injection and reduces local tissue reaction. Lehman and his associates have shown that combination with sodium thioacetate accomplishes the same end. Accordingly all diuretics recommended for parenteral use today are combined with either theophylline or thioacetate. Thiomerin, in which the organomercurial component is combined with thioacetate is the only one of the diuretics sufficiently nonirritating to be given subcutaneously. Even it has on occasion caused necrosis and sloughing of the skin. Except where the patient is to be instructed in the technique of subcutaneous self medication, the intramuscular route is advisable, even when using

overdigitalization, for they sensitize the myocardium to the actions of digitalis (see Chapter XIX).

TOXICITY

The toxicity of organic mercurial compounds may be conveniently considered under three major headings: (1) the toxic actions of mercury in organic combination; (2) hypersensitivity of the patient to the specific diuretic administered; and (3) manifestations of toxicity to loss of water and ions.

Toxic Actions of Organic Mercury. Local tissue reactions to intramuscular and subcutaneous injection and to rectal and oral administration have been considered under dose and route of administration. They are mitigated but by no means eliminated by inclusion of theophylline or thioacetate in the solutions for injection. As mentioned above, Neohydrin and Cumertilin are somewhat better tolerated on oral administration, largely because they are better absorbed and hence more effective in smaller dosage than the other drugs.

The most tragic of toxic manifestations is the immediate fatal reaction. It fortunately is rare, only 30 to 40 such accidents have been described, although it is probable that more deaths have occurred than have been reported. It has occurred only on intravenous administration and for this reason, the intravenous route should never be employed. The reaction occurs immediately and consists of pallor and cyanosis, a precipitous fall in blood pressure, substernal constriction, respiratory distress, cardiac irregularity, convulsions and death in ventricular fibrillation. The vast majority of patients succumbing have shown some premonitory signs and symptoms of toxicity on previous intravenous injections. In the experimental animal, large intravenous doses of mercurial diuretics commonly produce cardiac irregularities. Combination with monothiols markedly reduces cardiotoxicity, and Thiomerin is stated to be tolerated in cats in doses up to 160 times the acute lethal dose of nonthiol-containing drugs. Nevertheless, the fact that the diuretic response to intramuscular injection is equal to or better than to intravenous injection makes it inexcusable to subject the patient to the hazard of intravenous therapy even with Thiomerin.

Plasma Composition. Changes in plasma composition are generally conceded to be the result, not the cause, of diuresis. Due to more rapid loss of fluid in the urine than replacement from the interstitial reservoir, the concentration of protein in the plasma, and the hematocrit and viscosity of the blood increase. For this reason profound diuresis should be avoided in the elderly and in those with any thrombotic diathesis. Coronary thrombosis and cerebral artery thrombosis have occurred following massive diuresis. However, the complication is not a common one, no doubt because the edematous patient has a large reserve of extracellular fluid, and because constriction of blood volume tends to limit the diuretic response. Chloride is commonly lost in excess of fluid and in excess of sodium so that as the plasma concentration of chloride falls, bicarbonate increases and a more or less significant metabolic alkalosis develops. Such changes attain appreciable proportions in patients subjected to intensive diuretic therapy. As mentioned earlier they progressively limit the response to successive injections and are a cause of refractoriness in some patients (vide infra.)

If the patient responds well to diuretic therapy, the loss of sodium in the urine may also exceed to some extent the loss of water. As a consequence, plasma sodium may decrease slightly, perhaps from 140 to 135 mEq. per liter, More commonly, sodium is actively conserved, and potassium and ammonia balance a significant proportion of the urinary chloride. Under these circumstances plasma potassium falls, tissue stores of potassium decline, and sodium replaces some of the intracellular potassium. The degree of depletion of potassium depends on the duration of diuretic therapy and the extent to which potassium replaces sodium in the urine. It has long been known that edematous patients, optimally digitalized, when subjected to intensive diuretic therapy, may exhibit signs of digitalis toxicity. This has in the past been explained as due to concentration of digitalis in the body fluids in consequence of the loss of water in the urine. However, loss of potassium in the urine, depletion of body stores of potassium and hypopotassemia (hypokalemia) play far more significant roles in

origin and treatment of these conditions will be considered briefly in Chapter XIX.

POTENTIATION OF ACTION OF MERCURIAL DIURETICS

Acidifying Agents. It is now well recognized that the oral administration of an acidifying agent prior to the injection of a mercurial diuretic, greatly potentiates its action. Keith, Ethridge and many others have noted that ammonium chloride plus a mercurial diuretic causes a response considerably greater than the sum of the responses to the two agents given separately. Subsequently it has been observed that any agent which induces hyperchloremic metabolic acidosis potentiates the action of mercurial diuretics. Thus potentiation can be induced by the prior administration of ammonium chloride, calcium chloride, hydrogen or ammonium cycle ion exchange resins, and carbonic anhydrase inhibitors. Three explanations of potentiation have been advanced.

Mudge claims that acidosis is the significant factor, increasing the breakdown of the parent organomercurial compounds to liberate diuretically active mercuric ions in greater numbers. Increased blockade of sodium and chloride reabsorption results in enhanced urinary excretion.

Axelrod maintains that hyperchloremia is the significant factor, increasing the filtered load of chloride delivered into the renal tubules. If mercurial diuretics specifically inhibit some fraction of active reabsorption of chloride, as many believe, increasing the filtered load of chloride would cause increased urinary excretion of this ion.

The author agrees with Axelrod that hyperchloremia is the significant factor, but differs in holding that the proximal reabsorption of sodium is active whereas that of chloride is passive (see Chapter IV). In hyperchloremic acidosis, the quantity of chloride presented to the proximal tubules in the glomerular filtrate per unit time is increased relative to bicarbonate. Total sodium reabsorption remains essentially the same. Therefore, a greater than normal fraction of the sodium is reabsorbed with chloride, a lesser fraction with bicarbonate. Partial blockade of proximal sodium reabsorption by a mercurial diuretic will therefore deliver into the distal segment relatively more chloride and less bicarbonate than under

There is no doubt that large doses of mercurial diuretics in experimental animals produce signs of mercurialism not dissimilar to those produced by inorganic salts of mercury. The administration of excessive therapeutic doses, continued administration in the absence of an adequate diuretic response, administration to patients with severe renal insufficiently and marked nitrogen retention, all give rise to accumulation of drug in the body, and are potential causes of mercurialism. Manifestations of mercurialism include stomatitis, salivation, hemorrhagic colitis, and progressive renal failure. Mercurialism can be avoided if dosage is kept within a reasonable range and if the drugs are withheld in conditions known to be associated with accumulation. Many patients have received repeated injections of mercurials over periods of many years, deriving benefit from them without the development of adverse reactions.

Reactions of Hypersensitivity. Non-fatal and fatal toxic reactions due to an apparent anaphylactoid response to mercurial diuretics have been reported. These reactions usually occur one to two hours after administration. Asthmatic attacks and the development of acute pulmonary edema have been described. Other manifestation of drug idiosyncracy include dyspnea, substernal pain, cyanosis and circulatory collapse. Less grave signs of sensitivity include flushed skin, erythema morbilliformis, pruritis, urticaria, chills, fever, signs of bone marrow depression and exfoliative dermatitis. Changing the nature of the mercurial diuretic may ameliorate minor sensitivities. With more severe reactions, further therapy with mercurial diuretics should be avoided. Fortunately such reactions are relatively rare. Sensitization to thiomerin seems more common than to other drugs.

Manifestations of Toxicity Secondary to Loss of Ions and Water are by no means peculiar to mercurial diuresis. They may be encountered in therapy with any effective agent. They include acute circulatory collapse following a single profound diuresis, signs and symptoms of mild to severe hyponatremia following repeated diureses in patients maintained on a low salt regimen, and evidences of potassium depletion, including digitalis intoxication, in patients in whom dietary intake of potassium is inadequate. The

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The author agrees with Axelrod that hyperchloremia is the significant factor, but differs in holding that the proximal reabsorption of sodium is active whereas that of chloride is passive (see Chapter IV). In hyperchloremic acidosis, the quantity of chloride presented to the proximal tubules in the glomerular filtrate per unit time is increased relative to bicarbonate. Total sodium reabsorption remains essentially the same. Therefore, a greater than normal fraction of the sodium is reabsorbed with chloride, a lesser fraction with bicarbonate. Partial blockade of proximal sodium reabsorption by a mercurial diuretic will therefore deliver into the distal segment relatively more chloride and less bicarbonate than under

normal conditions. Distal reabsorption of sodium can be considered as the sum of two completely independent processes: (1) the exchange of sodium ions for either hydrogen, potassium, or ammonium ions and (2) the reabsorption of sodium and chloride as ion pairs. If the mechanism responsible for process (2) is limited with respect to sodium transport capacity and essentially saturated under normal conditions, it will be swamped under conditions of mercurial diuresis and even more overwhelmed under conditions of combined mercurial diuresis and hyperchloremia. Hyperchloremia would therefore be expected to potentiate mercurial diuresis.

Adrenal Steroids. It has been pointed out in Chapter XII that ACTH, cortisone, hydrocortisone, prednisone, and prednisolone may induce a primary diuresis in responsive patients, but more commonly potentiate the action of mercurial diuretics and carbonic anhydrase inhibitors. Possible causes of this potentiation are discussed in the chapter on steroid therapy.

Aminophylline. The administration of aminophylline in the course of mercurial diuresis may significantly enhance the response. There is general agreement that this type of potentiation is due, at least in part, to increased glomerular filtration rate. Increase in the filtered load of sodium and chloride, delivered into a nephron partially inhibited by a mercurial diuretic, results in increased excretion. Aminophylline also inhibits tubular reabsorption of sodium and chloride ions and the inhibition which it induces appears to be additive to that induced by the mercurial diuretic.

Resistance to Mercurial Diuretics. It has long been known that certain patients, early in the course of their disease, respond well to mercurial diuretics, but as time progresses, become less and less responsive to therapy, eventually becoming completely refractory. The patient is said to be mercury-fast. For many years mercury-fastness was considered synonymous with renal tolerance to mercury, presumably developed in response to low grade renal damage repeated with each succeeding dose of diuretic. This view no doubt had its origin in the observation on experimental animals that renal tubular epithelium, regenerated following near lethal doses of bichloride of mercury, is highly resistant to further insults with the heavy metal. In opposition to this thesis are the following

facts. Many patients continue to respond in adequate fashion to mercurial diuretics over periods of many years; a few, who are severely ill, fail to respond the first time the drugs are used. Often patients who are mercury-fast can be caused to regain responsiveness by altering the therapeutic regimen. Whether true renal tolerance ever develops is debatable; other factors play greater roles in mercury resistance.

Two factors are highly significant in the development of resistance to mercurial diuretics. First, as a result of progress of the basic disease or of intercurrent infection or complication, renal merchanisms of salt conservation are stimulated to such a degree that diuretics, as previously used, are no longer effective. Second, as a result of intensive diuretic therapy, changes in volume and composition of the body fluids occur which render diuretic therapy less than normally effective. In essence both statements imply that glomerulo-tubular imbalance has increased to such a degree that a formerly effective regimen no longer produces an adequate diuretic response

A reduction in glomerular filtration rate is one of the significant causes of glomerulo-tubular imbalance leading to inadeqate diuretic response. It may be a result of progress of the primary disease process, intercurrent infection, or pulmonary embolization and infarction In the cardiac, it may be a consequence of inadequate digitalization. It may be associated with the circulatory inadequacy of the low salt syndrome. It may be a sign of potassium depletion. In any event the filtered load of sodium and chloride is so reduced that essentially all is reabsorbed, even though some portion of the transport capacity of the tubules is depressed by the diuretic. Such patients are not merely mercury-fast they are diuretic-fast and fail to respond to any agent.

The filtered load of sodium and chloride is decreased by a reduction in plasma sodium and/or chloride concentration as well as by a reduction in filtration rate. Many believe that the hypochloremia which commonly attends intensive therapy with mercurial diuretics is a significant factor, the reduction in plasma chloride concentration reducing the filtered load. Others account for the effects of intensive diuretic therapy in terms of the

metabolic alkalosis which develops. They maintain that alkalosis stablizes the carbon-mercury bond of the diuretic molecule and reduces the availability of diuretically active mercuric ions.

Glomerulo-tubular imbalance is exaggerated by increased tubular reabsorption of sodium and chloride ions no less than by reduction in filtered load. Progress of the primary disease, infections, and complications increase the secretion of salt retaining steroids. Inadequate digitalization, and circulatory inadequacy due to the salt depletion, dehydration and hemoconcentration which may result from diuretic therapy are potent stimuli of aldosterone secretion.

Treatment of the Mercurial Resistant Patient. A variety of approaches to the therapy of edema in mercurial resistant patients is possible. One should always question the adequacy of digitalization in the patient with congestive failure who does not respond satisfactorily to mercurial diuretics despite adjuvant therapy with ammonium chloride. When increasing the dose of digitalis to the point of therapeutic response or toxicity, it is advisable to give oral potassium supplements in the form of 8 ounces of orange juice or 2 to 5 gm. of potassium chloride per day. Depletion of myocardial potassium may lead to arrhythmias and abnormalities of impulse conduction which may be interpreted as digitalis toxicity, even though the dosage of glycoside is less than optimum.

As pointed out above, ammonium chloride potentiates the action of mercurial diuretics and adequate dosage frequently causes the resistant patient to regain responsiveness. Ammonium chloride must be given in amounts and in a form which will induce hyperchloremic acidosis of significant proportions if it is to be effective. The required dose is 6 to 10 gm. or more per day. It should not be given in enteric coated tablets because of uncertain absorption. It should not be given in solutions more concentrated than 2.5 per cent because of gastric irritation. The daily dose should be divided equally and taken immediately before meals. A reasonable regimen is the following: 9 gm. of ammonium chloride per day for 6 days, on the fourth, fifth and sixth days, 2 ml. of a mercurial diuretic are given intramuscularly.

Some patients cannot tolerate doses of ammonium chloride adequate to produce a significant metabolic acidosis. These patients may be treated with a combination of ammonium chloride and acetazoleamide for 3 days. Because acetazoleamide interferes with mercurial diuresis, the drug is withdrawn two days prior to administration of a mercurial diuretic; the ammonium chloride is continued. On the fifth, sixth, and seventh days, 2 ml. of a mercurial diuretic are given intramuscularly. According to Luckey, this regimen is highly effective in mercurial resistant cardiacs. Ammonium chloride should not be given to patients with severe impairment of liver function, because of the danger of ammonia intoxication and hepatic coma, nor to patients with marked renal insufficiency, because of the danger of profound metabolic acidosis.

Weston has pointed out that the intravenous injection of 0.25 to 0.5 gm. of aminophylline 2 hr. after the intramuscular administration of a mercurial diuretic will frequently cause an adeqaute diuretic response in an otherwise refractory patient. Two hours is chosen to correspond to the peak diuretic effect of the mercurial. Aminophylline increases renal blood flow and filtration rate and increases the filtered load of ions delivered into the renal tubules. It also depresses renal tubular reabsorption of ions. Weston favors the oral adminstration of ammonium chloride for three days prior to combined mercurial and aminophylline therapy. He advises absolute confinement to bed for the period of diuretic action to obtain maximum benefit. The diuretic response to this regimen can be increased by elevation of the foot of the bed to a 30 degree angle and by application of elastic bandages to the edematous lower extremities from toes to thighs. This is especially useful in patients whose tissue turgor is low, namely in those who have been in failure repeatedly. The entire procedure should not be applied unless there is reason to believe that an adequate diuretic response will be obtained, because of the danger of precipitating pulmonary edema.

Others have suggested the slow intravenous infusion of as much as 4.0 ml. of a mercurial diuretic. This appears to the author to be hazardous at best, and doubly so in a patient who may well give an inadequate diuretic response. Diamox, given concurrently, tends

to reduce the diuretic response to a mercurial rather than to increase it. The actions of chlorothiazide and mercurial diuretics appear to be additive, perhaps synergistic. However, experience with combined therapy is limited.

Contraindications to Mercurial Diuretics. Mercurial diuretics are absolutely proscribed in acute renal failure and in acute nephritis, not only because of the danger of mercurial poisoning due to inadequate excretion, but also because of the possibility of aggravating the existing renal lesion. Some maintain that they should not be used in the nephrotic syndrome. The drugs are containdicated also in patients who have previously exhibited a toxic reaction or who have consistently failed to respond (cumulative toxicity). They may be used in chronic renal disease, if there is evidence of some renal reserve, i.e., if uremia does not develop as a consequence of dehydration.

SUMMARY

Mercurial diuretics act directly on the renal tubules to block a fraction of the reabsorption of sodium and chloride ions. Increased urine flow and loss of body weight occur in proportion to and are the osmotic consequences of loss of ions. Mercurial diuretics are highly and specifically concentrated in the renal cortex. They probably depress ion transport by forming inactive mercaptide complexes with sulfhydryl enzymes which supply energy to drive the reabsorptive machinery. It is uncertain whether the parent molecule has diuretic properties, or decomposition to mercuric ions is a requisite of diuretic action. It is equally uncertain whether mercurial diuretics block the active reabsorption of sodium or of chloride ions, although available evidence is readily interpretable in terms of the former thesis. Mercurial diuretics are rapidly eliminated from the body following parenteral adminstration and are secreted into the urine as complexes of cysteine or acetyl cysteine. They exert their major inhibitory effects on ion transport in the proximal tubule and are themselves most actively secreted in this same segment.

Clinically, mercurial diuretics may be administered by oral or intramuscular routes. In mild salt retaining states oral administra-

tion is frequently effective; more severely ill patients require intramuscular therapy. Intravenous injection is hazardous and no more effective than intramuscular injection, it should not be employed under any circumstances.

The efficacy of diuretic therapy can be considerably enhanced by the oral administration of ammonium chloride or other acidifying agents prior to injection of a diuretic; by the administration of aminophylline at the time of expected maximum diuretic action, by confinement to bed during the phase of diuresis; and by such ancillary procedures as elevation of the foot of the bed and application of elastic bandages to assist in the return of edema fluid to the circulation. Recently it has been shown that the administration of ACTH, cortisone, prednisone, and prednisolone increase the response to mercurial diuretics in resistant patients. Treatment of intercurrent infection, correction of potassium deficiency, and in the patient with congestive failure, adequate digitalization, improve the response. Mercurial diuretics are still the most generally effective agents available for the treatment of edema.

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Chapter XVII

SULFONAMYL DIURETICS

THE sulfonamyl compounds in use today as diuretics are unique therapeutic agents in that they were synthesized to block reversibly a specific renal enzyme, carbonic anhydrase. Indeed the only enzyme known to be inhibited by acetazoleamide (Diamox) and other similar unsubstituted monosulfonamyl compounds is carbonic anhydrase. Blockade of this enzyme reduces tubular reabsorption and increases urinary excretion of sodium, bicarbonate, and water. The recently introduced benzothiadiazine sulfonamyl compound, chlorothiazide (Diuril)[22] has, in addition to properties dependent on inhibition of carbonic anhydrase, the ability to depress tubular reabsorption of sodium and chloride ions. No doubt this action depends on inhibition of some other enzyme system which either serves as an ion carrier or supplies energy to a carrier mechanism. The sulfonamyl diuretics as a class are well absorbed, well tolerated, relatively non-toxic, and effective on oral administration. Accordingly, they fulfill several of the requirements of the ideal diuretic. However, they do not fulfill all, for they are frequently ineffective in the most severely ill patients. Furthermore, tolerance develops to some when they are given repeatedly, and all promote urinary loss of potassium and may deplete body reserves of this ion. However, these characteristics introduce no insurmountable difficulty in their use in properly selected patients.

The sulfonamyl compounds find their greatest use in the ambulatory patient for whom self medication is of greatest im-

[22]Hydrochlorothiazide (Hydrodiuril, Esidrix) is a recently synthesized derivative of chlorothiazide having no double bond in the heterocyclic ring. On a weight for weight basis it is claimed to be at least 5 times as potent as chlorothiazide. Whether it possesses any virtues over this latter drug as a therapeutic agent must await further tests

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capillary and alveolar walls and into the alveolar gas. It is obviously advantageous to accelerate the slow dehydration reaction enzymatically. As a matter of fact, enzyme is so abundantly present in red cells that this inherently slow reaction is no longer rate limiting. Instead, the chloride-bicarbonate ion shift across the erythrocyte membrane is probably the rate limiting step in the overall transformation and translocation of plasma bicarbonate into alveolar carbon dioxide Since the reverse sequence of reactions occurs during the brief interval an erythrocyte is in a tissue capillary, the advantage of accelerating the slow hydration reaction is again obvious.

Carbonic anhydrase is a zinc containing metalloprotein enzyme having a molecular weight of about 30,000 Mann and Keilin and later Krebs showed that its activity is strongly and specifically inhibited by sulfanilamide and other N^1 unsubstituted sulfonamides. The sulfonamides commonly used as chemotherapeutic agents are all N^1 substituted sulfonamyl compounds and have no anticarbonic anhydrase activity. The reaction of enzyme and inhibitor is simply described by the equation, $Enz + Inh \rightleftharpoons Enz \cdot Inh$. The affinity of enzyme for inhibitor is great, so the reaction is driven strongly to the right. Doses of acetazoleamide used in renal research on dogs commonly interfere in some degree with pulmonary excretion of carbon dioxide, resulting in an increased pCO_2 in arterial blood and a mild respiratory acidosis In contrast therapeutic doses in man do not induce significant respiratory acidosis. At least one reason for this difference between dog and man is the fact that acetazoleamide is highly concentrated within the erythrocytes of the dog, but to a lesser degree in the erythrocytes of man Chlorothiazide has little or no effect on pulmonary or tissue exchanges of carbon dioxide in either man or dog, for it is not specifically concentrated in red cells.

Carbonic Anhydrase Inhibitors as Diuretics. Shortly after the introduction of sulfanilamide as a chemotherapeutic agent, Southworth observed that the drug induces metabolic acidosis, associated with increased urine pH and increased urinary excretion of bicarbonate. A year or so later, Mann and Keilin demonstrated the fact, referred to above, that sulfanilamide and other N^1 unsubstituted

portance. Because their diuretic action is less drastic than that of organomercurials, it is easier to employ them over long periods in such fashion as to maintain a stable, edema-free state. They are especially useful in those patients who, on moderate salt restriction, require occasional injections of mercurial diuretics. Such patients can take a more liberal diet when on daily doses or intermittant courses of sulfonamyl diuretics, yet maintain themselves edema-free. Occasional patients, refractory to mercurial diuretics, respond with satisfactory diureses to these agents. Although chlorothiazide has been used for a relatively short time, clinical experience to date indicates that it is a more effective and more generally useful diuretic than acetazoleamide. Sulfonamyl compounds have a place in diuretic therapy of patients with congestive failure, cirrhosis, nephrosis, nephritis, premenstrual edema, and pre-eclampsia. Relatively speaking, they are benign therapeutic agents.

Nature of Carbonic Anhydrase. Carbonic anhydrase was first described by Roughton as an enzyme, present in erythrocytes, which greatly accelerates the attainment of equilibrum in the reversible reaction of carbon dioxide with water to form carbonic acid, (1) $CO_2 + H_2O \rightleftharpoons H_2CO_3$. The dissociation of carbonic acid to hydrogen and bicarbonate ions and the inverse association of these ions, (2) $H_2CO_3 \rightleftharpoons H^+ + HCO_3$, are ionic reactions, occur instantaneously, and are uninfluenced by the enzyme. Reaction (1) in an aqueous medium at body temperature is relatively slow, requiring approximately 200 seconds to come within 10 per cent of equilibrium. Reaction (1) is greatly speeded by carbonic anhydrase; the quantity of enzyme present in red cells is sufficient theoretically to accelerate the rate of reaction in whole blood 7,500-fold.

Most of the carbon dioxide produced in metabolic reactions is transported from tissues to lungs as bicarbonate ion in the blood plasma. In the second or so required for an erythrocyte to traverse a pulmonary capillary, bicarbonate ion must diffuse into the erythrocyte in exchange for chloride ion and associate with hydrogen ion to form carbonic acid; the carbonic acid must dehydrate to carbon dioxide and water; and the carbon dioxide must diffuse out of the red cell, across the plasma stream, through the

plays an important role in making hydrogen ions available in tubular cells at a rapid rate. In confirmation of this thesis they noted that sulfanilamide markedly depresses the rate of excretion of titratable acid.

Others have adopted the view that hydrogen ions are actively secreted by a redox ion pump in which the ferric iron of the cytochrome system oxidizes hydrogen atoms to hydrogen ions; $4\ Fe^{+++} + 4H \rightleftharpoons 4\ Fe^{++} + 4\ H^{+}$. The ferrous iron is then reoxidized as follows, to regenerate ferric iron and to produce equivalent numbers of hydroxyl ions, $4\ Fe^{++} + O_2 + 2\ H_2O \rightleftharpoons 4\ Fe^{+++} + 4\ OH^{-}$. The role of carbonic anhydrase in such a system would be that of neutralizing hydroxyl ions; $4\ OH^{-} + 4\ CO_2 \overset{ca}{\rightleftharpoons} 4\ HCO_3^{-}$. This system is one which has also been proposed as the mechanism of gastric secretion of acid. There is at the moment no definitive proof of either hypothesis. However, if the transfer of hydrogen ions into proximal tubular urine is passive and downhill along an electrochemical gradient as developed in Chapter IV, it is obvious that the first explanation of the role of carbonic anhydrase is correct. However, as was pointed out earlier, hydrogen ions must be actively transported in the distal tubule in exchange for sodium ions. The nature of the transport mechanism is unknown.

Subsequently Pitts and Lotspeich noted that sulfanilamide blocks a fraction of the reabsorption of filtered bicarbonate. They assigned this fraction to the distal tubule, pointing out that the exchange of hydrogen ions for sodium ions of a solution containing bicarbonate, would convert bicarbonate ions to carbonic acid. Dehydration of carbonic acid to CO_2 and water and back diffusion of CO_2 into renal capillary blood would be the equivalent of the reabsorption of bicarbonate ions per se. Roughly one-fifth of bicarbonate reabsorption was presumed to occur in the distal tubule by such a mechanism of ion exchange; four-fifths was presumed to occur in the proximal segment by a mechanism which specifically transports bicarbonate ion. This latter view is now known to be erroneous Proximal as well as distal bicarbonate reabsorption is probably effected by a process of Na^{+} for H^{+} exchange (*cf.* Figs 13, 14, 15B), but the demonstration of this fact

sulfonamides inhibit carbonic anhydrase activity in vitro. This, coupled with the observation of Davenport and Wilhelmi that the enzyme is highly concentrated in the renal cortex, paved the way for an explanation of the metabolic acidosis induced by sulfanilamide. Hoeber first suggested that renal carbonic anhydrase is in some way involved in the tubular reabsorption of bicarbonate, and that sulfanilamide, by inhibiting the enzyme, causes the excretion of alkaline urine and induces metabolic acidosis.

At the time two views were held as to the nature of the mechanism for acidifying the urine, both based on the premise that the acid, ultimately excreted as free titratable acid, enters the urine in the glomerular filtrate. According to one view, dibasic phosphate is preferentially reabsorbed from the tubular urine, leaving monobasic phosphate to be excreted as titratable acid. According to the other view, bicarbonate is preferentially reabsorbed, leaving carbonic acid to react with and to convert phosphate and other buffer salts into urinary titratable acid.

In 1945, Pitts and Alexander demonstrated that acidotic dogs, supplied with large amounts of buffer by the intravenous infusion of sodium phosphate or creatinine, excrete far more hydrogen ions in the urine than are present in the glomerular filtrate. They concluded that the renal tubular cells add hydrogen ions to the glomerular filtrate as it flows along the renal tubules. They suggested that tubular cells exchange hydrogen ions, generated within their substance by the dissociation of carbonic acid, for sodium ions in the tubular urine. The hydrogen ions and buffer anions are eliminated as titratable acid; the sodium and bicarbonate ions are reabsorbed into the peritubular capillaries to replenish the buffer reserves of the body fluids (*cf.* Fig. 15A). Since the kidneys of the dog can excrete titratable acid at a rate equivalent to 6,000 ml. of N/10 acid per day, it is obvious that an abundant source of H^+ ions must be available to tubular cells. Ultimately of course, the only possible source of hydrogen ions of this magnitude is water. Pitts and Alexander postulated that carbon dioxide is hydrated to carbonic acid within the acidifying cells of the distal tubule and that the carbonic acid so formed supplies hydrogen ions to the exchange mechanism. According to this view carbonic anhydrase

amino, or acylamino groups on the benzene ring increases enzyme inhibitory activity to the same order of magnitude as that of heterocyclic compounds. Dichlorphenamide (Daranide) is such a halogen substituted disulfonamyl compound. Combination of thiadiazine and benzene rings with 6 chloro, 7 sulfamyl substitution of

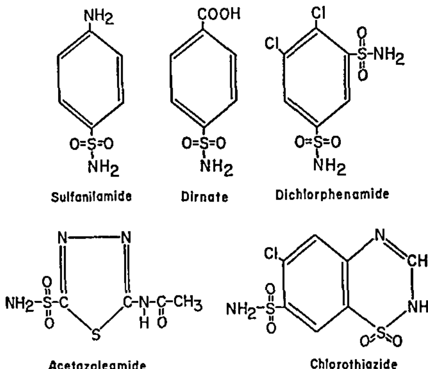


Fig 34. Structure of representative sulfonamyl diuretics.

the aromatic nucleus, as in chlorothiazide, introduces a new property, the ability to block sodium chloride reabsorption. According to Beyer, dichlorphenamide possesses this chloruretic characteristic in the same sense but to a lesser degree than chlorothiazide. Our own findings are opposed to this view. In the dog we have observed essentially no chloruresis with dichlorphenamide or acetazoleamide. Chlorothiazide, on the other hand, is very significantly chloruretic, certainly differs quantitatively and possibly qualitatively as well from the other two.

by Berliner required the development of carbonic anhydrase inhibitors several hundred times more potent than sulfanilamide.

It now became apparent to Schwartz that sulfanilamide might have potentialities as a diuretic. A study on patients in congestive failure demonstrated that this drug causes the excretion of an alkaline urine containing increased quantities of sodium, bicarbonate and potassium ions and that weight loss ensues. However, the required dosage was large and undesirable side reactions were marked. It was on the basis of these observations that Roblin and Clapp synthesized a series of highly active heterocyclic sulfonamide inhibitors of carbonic anhydrase, including acetazoleamide, in the hope of finding a clinically useful and orally effective diuretic.

CHEMICAL NATURE OF CARBONIC ANHYDRASE INHIBITORS

A common feature of the carbonic anhydrase inhibitors employed as diuretics is the unsubstituted sulfonamyl group, $—SO_2NH_2$. However, the nature of the molecule to which this group is attached greatly affects enzyme inhibitory and diuretic properties. Roblin and Clapp observed highest enzyme inhibitory action among heterocyclic compounds such as acetazoleamide (see Fig. 34), a compound which is some 300 times as potent in vitro as sulfanilamide. Even more potent is *benzothiazole — 2 — sulfonamide*, some 800 times as active as sulfanilamide. However, in vivo it is completely devoid of diuretic properties due to rapid metabolism and conjugation. *Methazoleamide*, in which a methyl group is attached to one of the heterocyclic ring nitrogens of acetazoleamide is a highly active enzyme inhibitor, penetrates eye and brain rapidly and accordingly is a potent antiglaucoma and anticonvulsant drug. However, it possesses no virtues as a diuretic over acetazoleamide.

The aromatic monosulfonamyl compounds such as sulfanilamide and Dirnate have a relatively low order of activity. The latter, after brief exploitation as a diuretic, has been withdrawn from use. Sprague, Beyer and their associates have observed a high order of activity among aromatic disulfonamyl compounds, *especially* those of 1, 3 configuration. Furthermore, substitution of halogen,

suppresses a different series of reactions which furnish some 10 per cent of the energy. Since their actions are additive, the two drugs together suppress about 30 per cent of sodium and chloride transport. There remains, however, in the doubly blocked renal tubules from 60 to 70 per cent of normal ion transport capacity. The energy for this moiety is presumably supplied by metabolic reactions not sensitive either to mercurial diuretics or chlorothiazide.

RENAL ACTIONS OF SULFONAMYL DIURETICS

Acetazoleamide. Maren has described the diuretic effects of acetazoleamide in normal dogs in the following terms. When the drug is given in a single daily dose of 5 to 10 mg. per Kg., it promptly alkalinizes the urine. The excretion of sodium, potassium, and bicarbonate is increased, that of ammonia and titratable acid is sharply reduced. The duration of action is relatively short (6 hr. or less), and during the remainder of the day the losses of ions are made up from dietary intake, and the acidosis which results from bicarbonate loss is corrected by enhanced ammonia and titratable acid excretion. Under such conditions, the drug exerts a more or less constant action from day to day; i.e., tolerance does not develop. However, if dosage is increased and especially if multiple doses are administered, initial ion losses are greater, effects are more prolonged, and after a period, the animal becomes refractory to the drug.

Table XIII summarizes an experiment of Pitts *et al.* which illustrates the acute response of a dog to a relatively large dose of acetazoleamide administered intravenously. The animal was prehydrated with saline in order that the diuresis might not be curtailed by depletion of extracellular fluid reserves. The first two collection periods describe control observations; the succeeding four, describe observations made immediately following the intravenous administration of 10 mg. per Kg. of acetazoleamide as a priming dose and the addition of drug to the infusion in amounts sufficient to provide 15 mg. per Kg. per hr. Little consideration should be accorded urine flow in such experiments as these, for it is the least reliable index of diuretic activity.

Enzyme Inhibitory Actions of Sulfonamyl Compounds. The heterocyclic and aromatic monosulfonamyl compounds and the aromatic disulfonamyl compound, dichlorphenamide, exhibit a major, if not a single, enzyme inhibitory action, namely, that of blocking carbonic anhydrase. The concept of Roblin that enzyme inhibition results from steric similarities between the sulfonamyl group and carbonic acid is illustrated in Figure 35. The inhibitor

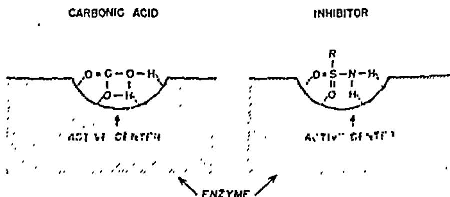


Fig. 35. Concept of Dr. R O Roblin of the mechanism of action of carbonic anhydrase inhibitors based on steric similarities between carbonic acid and the unsubstituted sulfonamyl group. (From J. M Sprague· *Ann. New York Acad. Sc.*, 71:321, 1958.)

has a stronger affinity for the active sites on the enzyme molecule than does carbonic acid so that the later is displaced and enzymatic activity abolished.

Chlorothiazide probably possesses an enzyme inhibitory action in addition to that of blocking carbonic anhydrase. The nature of the enzyme affected is unknown, except that it is concerned with the renal tubular reabsorption of sodium and chloride. Chlorothiazide blocks sodium chloride transport to a significant degree, but whether by interfering with a carrier mechanism or with some metabolic process which supplies energy to a carrier mechanism is uncertain. Pitts and his associates favor this latter view. They suggest that chlorothiazide, like organomercurial compounds, interferes with the sodium pump of proximal tubular cells. Mercurials suppress metabolic reactions which furnish roughly 20 per cent of the energy required to drive the pump; chlorothiazide

It is apparent that the drug reduced plasma pH and increased urine pH within the first 10 minutes after the start of intravenous infusion. The decrease in plasma pH resulted from an increase in the pCO_2 of arterial blood due to interference with liberation of CO_2 in the lungs. The increase in urine pH resulted from a marked increase in excretion of bicarbonate, from control values of 32 and 17 μEq. per min. to well over 400 μEq. per min. Increased excretion of bicarbonate was due to depression of tubular reabsorption from 99 per cent of that filtered to 64 per cent. The increased urinary bicarbonate was balanced in part by sodium and in part by potassium. Chloride excretion was little changed either in terms of absolute rate of excretion or in terms of per cent of the filtered moiety reabsorbed.

It should be remembered that potassium is both reabsorbed from and secreted into the tubular urine. Apparent depression of tubular reabsorption from some 90 per cent to 14 to 40 per cent may actually represent an increase in tubular secretion. Berliner believes that reabsorption of potassium is nearly complete in the proximal tubule, and that variations in excretion, such as those induced by acetazoleamide, are in truth variations in tubular secretion of potassium in the distal segment.

In the experiment summarized in Table XIII, the dog was moderately acidotic in the control periods (plasma pH 7.3; plasma $BHCO_3$, 18 mEq. per liter). This acidosis, metabolic in nature, was dilutional in origin, and due to the large amount of saline administered prior to the start of the experiment. Had the plasma bicarbonate been more within the normal range (24 to 28 mEq. per liter), urinary losses of bicarbonate would probably have been greater.

Dichlorphenamide is in many respects similar in its renal actions to acetazoleamide, depressing reabsorption and increasing urinary excretion of sodium, potassium and bicarbonate ions. Due to increased excretion of bicarbonate, the urine becomes alkaline. According to Beyer, dichlorphenamide exerts a more prolonged and profound depression of ion reabsorption at comparable dose levels, is more active under conditions of low salt intake, and is less subject to the development of tolerance than acetazoleamide.

TABLE XIII

THE EFFECTS OF A LARGE DOSE OF ACETAZOLEAMIDE ON REABSORPTION AND EXCRETION OF IONS BY THE DOG

Total Elapsed Time	Urine Flow	Glom. Filt. Rate	pH		Plasma Concentration				Rate of Excretion				Per Cent of Filtered Reabsorbed			
			Plasma	Urine	Na	K	Cl	HCO_3	Na	K	Cl	HCO_3	Na	K	Cl	HCO_3
(min.)	(ml. per min.)				(milliequivalents per liter)				(microequivalents per minute)							
						Dog wt. = 21 Kg.										
0-10	3.9	109	7.30	6.76	149	3.50	123	18.5	882	23	843	32	94.3	93.8	94.0	98.7
10-20	4.2	98.4	7.32	6.50	150	3.66	124	18.1	808	29	731	17	94.3	91.5	94.3	99.0
20	Acetazoleamide: 10 mg. per Kg., Prime; 15 mg. per Kg. per hr., Infusion															
20-30	5.0	82.9	7.20	7.58	150	3.39	122	16.0	980	230	720	445	91.8	18.1	93.1	68.0
30-40	5.5	86.0	7.22	7.59	150	3.22	122	15.2	1150	142	736	488	90.7	46.0	93.3	64.3
-50	5.2	76.4	7.21	7.56	148	3.24	125	16.3	1060	130	655	468	90.0	44.7	93.4	64.2
-60	4.2	78.7	7.22	7.61	147	3.04	123	16.0	800	160	679	434	92.6	29.5	93.3	67.1

(From R. F. Pitts, F. Krück, R. Lozano, D. W. Taylor, O. P. A. Heidenreich, and R. H. Kessler: *J. Pharmacol. & Exper. Therap.*, 123:89, 1958.)

It is apparent that the drug reduced plasma pH and increased urine pH within the first 10 minutes after the start of intravenous infusion. The decrease in plasma pH resulted from an increase in the pCO_2 of arterial blood due to interference with liberation of CO_2 in the lungs. The increase in urine pH resulted from a marked increase in excretion of bicarbonate, from control values of 32 and 17 μEq. per min. to well over 400 μEq. per min. Increased excretion of bicarbonate was due to depression of tubular reabsorption from 99 per cent of that filtered to 64 per cent. The increased urinary bicarbonate was balanced in part by sodium and in part by potassium. Chloride excretion was little changed either in terms of absolute rate of excretion or in terms of per cent of the filtered moiety reabsorbed.

It should be remembered that potassium is both reabsorbed from and secreted into the tubular urine. Apparent depression of tubular reabsorption from some 90 per cent to 14 to 40 per cent may actually represent an increase in tubular secretion. Berliner believes that reabsorption of potassium is nearly complete in the proximal tubule, and that variations in excretion, such as those induced by acetazoleamide, are in truth variations in tubular secretion of potassium in the distal segment.

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			Plasma	Urine	Na	K	Cl	HCO_3	Na	K	Cl	HCO_3	Na	K	Cl	HCO_3
(min)	(ml. per min.)				(milliequivalents per liter)				(microequivalents per minute)							
Dog wt. = 21 Kg.																
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40-50	5.2	76.4	7.21	7.56	148	3.24	125	16.3	1060	130	655	468	90.0	44.7	93.4	64.2
50-60	4.2	78.7	7.22	7.61	147	3.04	123	16.0	800	160	679	434	92.6	29.5	93.3	67.1

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TABLE XIV

THE EFFECTS OF A LARGE DOSE OF CHLOROTHIAZIDE ON REABSORPTION AND EXCRETION OF IONS BY THE DOG

Total Elapsed Time	Urine Flow	Glom. Filt. Rate	pH		Plasma Concentration				Rate of Excretion				Per Cent of Filtered Reabsorbed			
			Plasma	Urine	Na	K	Cl	HCO_3	Na	K	Cl	HCO_3	Na	K	Cl	HCO_3
(min)	(ml per min)				(milliequivalents per liter)				(microequivalents per minute)							
Dog. wt. = 18 Kg.																
0-10	4.7	91.7	7.30	6.06	146	3.75	122	19.2	560	25	590	5	95.5	92.5	95.0	99.8
10-20	3.9	92.1	7.33	5.98	147	3.64	120	18.4	550	26	560	3	95.8	91.8	95.0	99.9
20 *Chlorothiazide: 10 mg. per Kg., Prime; 15 mg. per Kg per hr., Infusion*																
20-30	7.1	84.2	7.33	7.04	147	3.75	121	17.9	1120	68	1020	160	90.5	77.3	90.4	90.0
30-40	8.2	82.6	7.33	7.27	147	3.64	120	17.8	1290	147	1150	204	88.8	49.3	88.9	86.8
-50	7.2	75.9	7.32	7.26	147	3.63	124	17.7	1090	126	1010	160	89.6	51.9	89.8	88.7
-60	6.7	75.5	7.30	7.23	148	3.63	122	17.9	1070	111	1010	143	89.9	57.4	89.5	90.0

(From R. F. Pitts, F. Kruck, R. Lozano, D W. Taylor, O P. A. Heidenreich, and R. H. Kessler: *J. Pharmacol. & Therap.* 123:89, 1958)

Since the two drugs inhibit carbonic anhydrase in vitro to nearly the same degree, greater in vivo activity of dichlorphenamide might result from greater concentration in, or more ready penetration of renal tissue. Such properties have not been demonstrated. It is claimed that dichlorphenamide depresses reabsorption and increases excretion of chloride to a significantly greater extent than does acetazoleamide.

Chlorothiazide. Beyer and his associates have shown that chlorothiazide exhibits properties both of carbonic anhydrase inhibitors and of mercurial diuretics. Due to the free sulfonamyl group, it inhibits carbonic anhydrase in vitro, and in relatively large doses in vivo, alkalinizes the urine, depresses the reabsorption of bicarbonate, and promotes the secretion of potassium. In addition it causes natriuresis and chloruresis, actions similar to those of mercurial diuretics. In fact in the usual orally effective doses, the major response is natriureses and chloruresis. The urine may fail to become alkaline, although potassium excretion is enhanced. However, the basic mechanisms of chloruretic action of chlorothiazide and of the organomercurial compounds differ in the sense that their actions are additive when the two are given together, each in maximally effective dosage. Furthermore, BAL (dithiopropanol) blocks the action of mercurial diuretics, whereas it has no effect on chlorothiazide While chlorothiazide can induce diuresis in the presence of fluid retention induced by corticosteroids, it is not a competitive antagonist in the sense of the antialdosterones, for it enhances rather than reduces the potassium loss characteristically induced by these steroids.

Table XIV summarizes an experiment of Pitts et al which illustrates the acute response of a dog to a relatively large dose of chlorothiazide administered intravenously. The animal was prehydrated in exactly the same manner employed in the experiment summarized in Table XIII. The two experiments are, therefore, as nearly comparable as any two are likely to be on different mongrel dogs.

It is apparent that the drug increased urine pH within the first 10 mins. after intravenous administration, yet had no discernable effect on plasma pH. The increase in urine pH resulted from a

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…d …ed …e	Urine Flow	Glom. Filt Rate	pH		Plasma Concentration				Rate of Excretion				Per Cent of Filtered Reabsorbed			
			Plasma	Urine	Na	K	Cl	HCO_3	Na	K	Cl	HCO_3	Na	K	Cl	HCO_3
…)	(ml. per min)				(milliequivalents per liter)				(microequivalents per minute)							
								Dog. wt. = 18 Kg.								
…0	4.7	91.7	7.30	6 06	146	3.75	122	19.2	560	25	590	5	95.5	92.5	95 0	99 8
…0	3.9	92.1	7.33	5 98	147	3.64	120	18.4	550	26	560	3	95.8	91.8	95.0	99.9
…0 Chlorothiazide: 10 mg. per Kg., Prime; 15 mg per Kg per hr., Infusion																
…0	7 1	84 2	7.33	7 04	147	3.75	121	17.9	1120	68	1020	160	90.5	77.3	90 4	90 0
…0	8 2	82 6	7.33	7.27	147	3.64	120	17 8	1290	147	1150	204	88 8	49.3	88 9	86 8
…0	7 2	75 9	7 32	7 26	147	3 63	124	17.7	1090	126	1010	160	89.6	51.9	89 8	88 7
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It is apparent that the drug increased urine pH within the first 10 mins. after intravenous administration, yet had no discernable effect on plasma pH. The increase in urine pH resulted from a

significant increase in excretion of bicarbonate, from control values of 5 and 3 μEq. per min. to as much as 200 μEq. per min. Even more striking was the chloruresis induced by the drug, the rate of excretion of chloride increasing from 560 μEq. per min. to over 1000 μEq. per min. The increase in excretion of chloride was roughly balanced by an equivalent increase in excretion of sodium. Both chloruresis and natriuresis resulted from depression in tubular reabsorption, from 95 per cent of that filtered to 89 per cent. Reabsorption of potasisum was diminished and excretion enhanced. The same reservations with respect to the meaning of diminished potassium reabsorption, as were outlined above for acetazoleamide, apply to chlorothiazide. Again the moderate dilutional metabolic acidosis reduced in some degree the extent of bicarbonate excretion in comparison with that which would have obtained had the plasma level been within a more normal range.

Comparison of Renal Actions of Acetazoleamide, Dichlorphenamide, and Chlorothiazide. In vitro, acetazoleamide and dichlorphenamide are significantly more potent inhibitors of carbonic anhydrase than is chlorothiazide. This is reflected in a more pronounced inhibition of reabsorption and a greater increase in excretion of bicarbonate following acetazoleamide and dichlorphenamide than chlorothiazide. A somewhat different but related expression of carbonic anhydrase inhibition is promotion of urinary potassium loss. Again the activity of acetazoleamide and dichlorphenamide exceeds that of chlorothiazide. Still a third expression of this property is depression of blood pH due to interference with CO_2 elimination in the lungs. Acetazoleamide is the most active of the drugs in this respect. Chlorothiazide possesses a property which distinguishes it quantitatively and probably qualitatively as well from acetazoleamide and dichlorphenamide, namely, the ability to block the reabsorption of sodium and chloride ions.

From the above noted considerations, one would predict that chlorothiazide would be a more effective diuretic for clinical use than either acetazoleamide or dichlorphenamide, i.e., it would cause less disturbance in electrolyte pattern of the body fluids, it would cause less depletion of body stores of potassium, and it would cause greater loss of sodium and chloride ions. All three

drugs depress glomerular filtration rate when administered intravenously in large doses. However, when given orally in the usual therapeutic doses, they have no significant effect on glomerular function.

SITE OF ACTION OF ACETAZOLEAMIDE, DICHLORPHENAMIDE, AND CHLOROTHIAZIDE

Acetazoleamide. If one neglects depression of glomerular filtration rate which commonly results when large doses of acetazoleamide are given intravenously, all renal actions of this drug can be explained in terms of a specific depression of mechanisms for exchange of hydrogen ions for sodium ions. This action, exerted on proximal tubules, reduces reabsorption of bicarbonate (*cf.* Fig. 13). Exerted on distal tubules, it further reduces bicarbonate reabsorption and inhibits acidification of the urine (*cf.* Fig. 15 A,B). Since the urine becomes alkaline, the distal secretion of ammonia is reduced (*cf.* Fig. 15C). The mechanism for hydrogen and sodium exchange is also concerned with potassium and sodium exchange. The overall activity of this joint mechanism in reabsorbing sodium is only moderately depressed by acetazoleamide. Accordingly, blockade of hydrogen exchange by the drug facilitates potassium exchange, and exhibition of acetazoleamide in large doses frequently causes net potassium secretion. The drug has little effect on other renal transport mechanisms, such as reabsorption of glucose and secretion of para-aminohippurate.

Figure 36 illustrates both the nature and the localization within the nephron of these actions of acetazoleamide. These data were derived from an experiment on a dog utilizing the "stop flow" method of localizing tubular functions described in Chapter XVI. The experiment was performed in two parts, the first consisted of a control series of observations, the second consisted of a series following the administration of 10 mg. per Kg. of acetazoleamide as a priming dose and the addition of the drug to the infusion in amounts sufficient to provide 15 mg. per Kg. per hr. In each of the two series of observations the ureteral catheter was clamped for a period of four minutes.

If one surveys Figure 36 from top to bottom, the significant

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If one surveys Figure 36 from top to bottom, the significant

actions of acetazoleamide are readily apparent. The control experiment is identified by circles, the acetazoleamide experiment by triangles. Urine samples obtained from the distal part of the nephron, i.e. those to the right of the graph, were acidified from a baseline value of pH 8.0[23] to roughly pH 5.0 in the control experiment. Following acetazoleamide, distal acidification was blocked. In the control experiment, ammonia was secreted into the distal urine samples, in highest concentration at the site of maximum acidification. Following acetazoleamide, and as a direct consequence of failure of acidification, ammonia secretion was inhibited. In contrast, the distal secretion of potassium, relatively slight in the control experiment, was greatly exaggerated by acetazoleamide.[24] Distal reabsorption of sodium was relatively unaffected by the drug.

This experiment graphically portrays the reciprocal relationship between the exchange of hydrogen and ammonium ions or potassium ions for sodium, and illustrates the fact that a carbonic anhydrase inhibitor, by depressing distal hydrogen and ammonia exchange, facilitates potassium exchange. It should be pointed out that distal reabsorption of sodium ($U/P_{Na}/U/P_{Cr} < 0.05$) can by no means be ascribed solely to potassium, hydrogen and ammonia exchange. Simple calculations indicate that most of the sodium is reabsorbed in the distal segment in association with chloride ions Only a small, albeit significant, fraction of the total is exchanged for H^+, K^+ and NH_4^+.Furthermore, it is apparent that the site of most avid reabsorption of sodium is slightly more proximal than

[23]Since it was impossible with the experimental techniques employed to prevent loss of CO_2, all specimens were equilibrated with air prior to measurement of pH. This exaggerates pH differences, for it causes those samples which contain bicarbonate (proximal) to become more alkaline, whereas those with negligible bicarbonate (distal) do not change reaction.

[24]The high $U/P_K/U/P_{Cr}$ values in the proximal tubule cannot be interpreted with any degree of assurance. The ratio of 1 5 to 2.0 might be considered as indicating potassium secretion in the proximal segment. However, samples held in contact with proximal epithelium for the 4 min. period of clamping must pass through the distal segment en route to collection. It is, therefore, probable that much of the potassium in these samples was added during transit through the distal part of the nephron Any attempt to quantify a proximal contribution to potassium excretion is so dependent on assumptions as to render it of doubtful value.

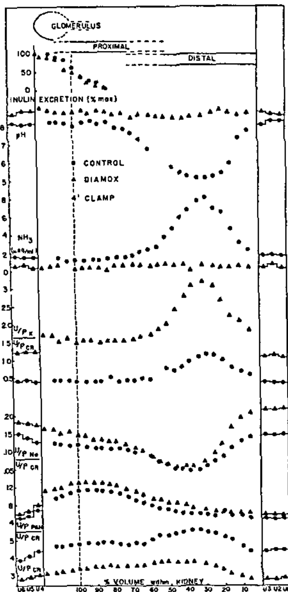


Fig. 36. "Stop-flow" experiments on a dog which localize inhibition of urine acidification and of ammonia secretion and stimulation of potassium secretion by acetazoleamide to the distal nephron. The data indicate equivocal depression of proximal sodium reabsorption and negligible depression of distal sodium reabsorption. See Chapter XVI, page 232, for description of stop flow method. (From R. F. Pitts, R. S. Gurd, R. H. Kessler, and K. Hierholzer *Am J. Physiol*, *194*:125, 1958)

the site of maximum exchange of sodium for hydrogen, ammonia, and potasisum.

One additional finding apparent in Figure 36 possibly has significance. Values of $U/P_{Na}/U/P_{Cr}$ in specimens from the proximal segment are uniformly higher after acetazoleamide than in control experiments; i.e., proximal reabsorption of sodium is inhibited to some extent by the drug. It is probable that the inhibited fraction is that ordinarily reabsorbed in exchange for hydrogen and thus represents sodium bicarbonate. The effect is small because sodium bicarbonate reabsorption accounts for no more than one-fifth of total proximal sodium reabsorption and the dose of acetazoleamide is by no means adequate to block all.

Dichlorphenamide produces the same alterations in renal function at the same sites within the nephron as does acetazoleamide. Figure 37 describes a control experiment and an experiment performed immediately after the administration of dichlorphenamide. Both the control and dichlorphenamide experiments were performed on the same kidney of the same dog. It is evident that dichlorphenamide blocks ammonia secretion and acidification of the urine, and enhances the secretion of potassium, all functions of the distal portion of the nephron. The drug has little effect on sodium or chloride reabsorption in either the proximal or distal nephron. It is impossible in experiments such as these to distinguish between the actions of dichlorphenamide and acetezoleamide, if indeed any differences exist.

Chlorothiazide. Figure 38 illustrates both the nature and localization within the nephron of the actions of chlorothiazide. The data were derived from an experiment identical with those of Figures 36 and 37, except that following the control series of observations, chlorothiazide was administered intravenously in the same dosage employed for acetazoleamide. Since the dose of chlorothiazide was large in comparison with that administered clinically, the qualitative and even quantitative manifestations of carbonic anhydrase inhibition are essentially the same as those following acetazoleamide and dichlorphenamide. Thus depression of ammonia secretion, depression of urine acidification, and enhancement of potassium secretion in the distal nephron are essen-

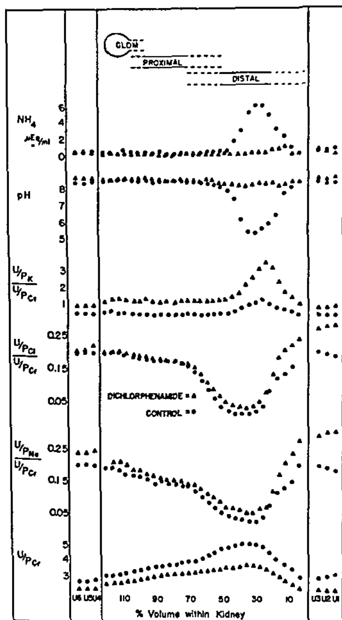


Fig 37. "Stop-flow" experiments on a dog which localize inhibition of urine acidification and of ammonia secretion and stimu-

Pitts- *Am. J. Physiol.*, *196*:1346, 1959.

tially as complete following chlorothiazide as acetazoleamide or dichlorphenamide. The distal reabsorption of sodium and especially the distal reabsorption of chloride are little if at all affected by chlorothiazide. In this respect also chlorothiazide is similar to acetazoleamide and dichlorphenamide. However, proximal reabsorption of sodium is much more significantly depressed by chlorothiazide than by the other two diuretics. Furthermore, it is evident that proximal depression of sodium reabsorption is associated with a nearly equivalent depression of chloride reabsorption. Proximal reabsorption of bicarbonate may well be depressed by chlorothiazide but is impossible to identify this action because of the greater effect on chloride. It is obvious that chlorothiazide is secreted by the proximal tubules, the $U/P_{Ctz}/U/P_{Cr}$ ratio attaining values as high as 11.0. The mechanism which secretes chlorothiazide is the same as that which secretes paraaminohippurate, penicillin, diodrast, phenol red, etc.

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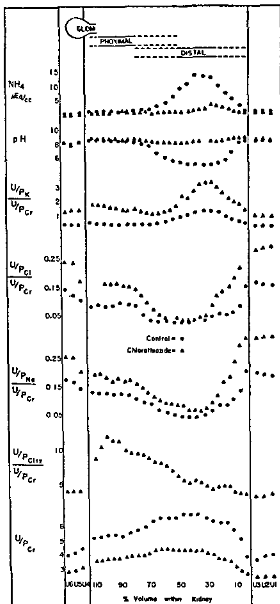


Fig 38 "Stop-flow" experiments on a dog which localize inhibition of urine acidification and of ammonia secretion and stimulation of potassium secretion by chlorothiazide to the distal nephron. The data indicate significant depression of reabsorption of sodium and chloride ions in the proximal nephron (From R. H. Kessler, K. Hierholzer, R S. Gurd, and R. F. Pitts· *Am. J. Physiol*, *196*·1346, 1959.

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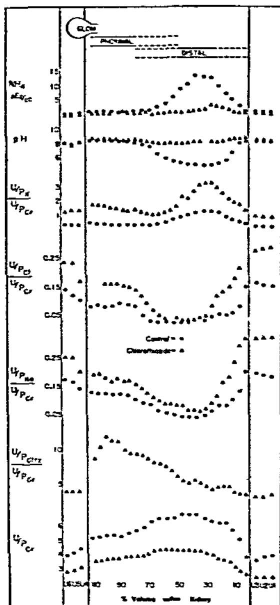


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is exchanged for sodium and drained from tissues. Hypopotassemia and depletion of cellular stores of potassium result.

The extent of the changes in plasma and tissue composition depends on the magnitude and frequency of the dose, on the nature of the drug administered and on the adequacy of dietary intake of ions. Since acetazoleamide and dichlorphenamide are more powerful inhibitors of carbonic anhydrase than is chlorothiazide, significant aberrations in body fluid composition are more prone to follow their use. However, significant potassium depletion occurs when chlorothiazide is administered in repeated doses if dietary intake is inadeqaute. Acidosis is less a problem with chlorothiazide than with acetazoleamide and dichlorphenamide. In fact certain patients develop a mild hypochloremic alkalosis while on continued therapy with chlorothiazide due to perferential loss of sodium and chloride ions.

Drug Refractoriness with respect to effects on excretion of sodium and bicarbonate ions derives in part from the above mentioned changes in plasma composition. Metabolic acidosis, resulting from urinary loss of bicarbonate, reduces the filtered load of bicarbonate delivered into the renal tubules. Even though availability of hydrogen ions to the proximal and distal exchange mechanisms is reduced by carbonic anhydrase inhibitors, the uncatalyzed rate of hydration of carbon dioxide to carbonic acid may be adequate to provide for the complete reabsorption of a reduced amount of filtered bicarbonate. Accordingly, depression of reabsorption of sodium bicarbonate is self-limited. It is claimed that development of refractoriness to dichlorphenamide is much less a limiting factor in its use as a diuretic than is that to acetazoleamide. Why this should be is not clear to the author. Since the major therapeutic effect of chlorothiazide is depression of sodium and chloride reabsorption, refractoriness in the sense described does not occur.

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According to Beyer, chlorothiazide is rapidly absorbed from the gut. It appears in the blood stream in determinable amounts within 15 min. after an oral dose, reaches peak concentrations within 45 min., and declines over 6 hr. or more. On an average, 50 per cent of an oral dose is excreted in 6 hr.; over 95 per cent of an intravenous dose is eliminated in the same period of time. The renal clearance of chlorothiazide is high, approximating effective renal plasma flow. As pointed out above, it is secreted by the same renal tubular mechanism which secretes paraaminohippurate, diodrast, phenol red, and penicillin. However, secretory transport may be dissociated from diuretic activity by the administration of probenecid, a drug which blocks tubular transport without affecting diuretic activity. Little or no chlorothiazide penetrates muscle, fat, brain or aqueous humor. However, it is secreted into the bile in appreciable amounts following oral administration. In contrast to acetazoleamide, chlorothiazide is not concentrated in erythrocytes, cellular concentrations in general are less than plasma concentration and drug is not retained in any tissue. Acetazoleamide, dichlorphenamide, and chlorothiazide all exert their major diuretic effects within the first 6 hrs. after oral administration.

Alterations in Body Fluid Composition. Characteristically carbonic anhydrase inhibitors depress plasma pH; first, because they interfere with pulmonary excretion of carbon dioxide (in the dog more than in man, and acetazoleamide more than chlorothiazide); second, because they induce urinary excretion and reduce plasma concentration of bicarbonate. They likewise promote urinary loss of postassium, presumably by interfering with the supply of hydrogen ions to the exchange mechanism of distal tubular cells. Lacking hydrogen ions, potassium ions are substituted; potassium

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A somewhat less desirable regimen is the administration of 0.25 to 0.5 gm. t.i.d. for a period of 2 to 3 days, whereupon the average patient becomes refractory. The drug is withheld for 2 to 3 days, and the course repeated. Correction of acidosis and excretion of chloride occurs in the drug free interval. Where production of hyperchloremic acidosis as a means of potentiating mercurial diuretics is the desired end, Luckey advises the daily administration of 0.75 gm. of acetazoleamide and up to 10 gm. of ammonium chloride in divided doses for 3 days; acetazoleamide is withdrawn but ammonium chloride is continued on the 4th and 5th days; and 2 ml. of Mercuhydrin are given on the 5th, 6th, and 7th days. This regimen has been particularly effective in diuretic resistant patients in congestive failure. Most agree that the administration of acetazoleamide and a mercurial diuretic on the same day reduces the response obtained with either agent. Luckey finds the peak diuretic response to mercury 48 hr. after the last dose of acetazoleamide. Where acetazoleamide is administered for its primary diuretic action, ammonium chloride is of course contraindicated, for it merely hastens the development of refractory acidosis.

It has been pointed out in Chapter XII on steroid therapy that the administration of ACTH, cortisone, hydrocortisone, prednisone, and prednisolone potentiate the diuretic actions of both organomercurial and sulfonamyl compounds. The mechanism of this potentiation is by no means clear. However, the administration of prednisone or prednisolone increases the response to acetazoleamide in the patient otherwise refractory to the drug. It seems likely that these steroids would also potentiate the action of dichlorphenamide and chlorothiazide.

Dichlorphenamide has been little studied clinically. Hence there is insufficient experience upon which to base dosage and therapeutic regimen. It is probable that this drug should be employed in much the same manner as acetazoleamide.

Chlorothiazide is ordinarily administered in a total daily dose of 0.5 gm. to 2.0 gm., in the lower range as a single dose, in the

anorexia or dietary fad, potassium may be drained from tissues to urine. Cells become depleted of potassium and gain sodium. Abdominal distension, weakness, lassitude, and cardiac irregularities develop. Whenever carbonic anhydrase inhibitors are used, it is wise to ensure an adequate potassium intake, e.g., 8 ounces or more of orange juice or 2 to 5 gm. of KCl per day.

All refractoriness to carbonic anhydrase inhibitors, does not derive from metabolic acidosis. Undoubtedly a significant factor is the reduction in glomerular filtration rate which occurs in response to a reduction in extracellular volume. Reduction in extracellular volume no doubt stimulates aldosterone secretion and enhances tubular reabsorption of sodium. Both responses increase glomerulo-tubular imbalance and favor retention of salt and water. The hypokalemia which develops in response to continued exhibition of a potent carbonic anhydrase inhibitor seems actually to stimulate the distal tubular mechanisms for acidification of the urine and for ammonia secretion, and hence contributes to the development of refractoriness.

Dosage and Route of Administration. Acetazoleamide, dichlorphenamide, and chlorothiazide are readily and rapidly absorbed on oral administration. While it is true that diuretic responses are more immediately impressive when a given dose is administered intravenously than when administered orally, one of the major virtues of these agents is lost if one resorts to parenteral therapy. If response to oral therapy is inadequate, it is better to turn to more potent agents rather than to intravenous administration.

Acetazoleamide may be administered in one of two ways to avoid, in whatever degree possible, the development of drug tolerance. A dose of 0.25 to 0.75 gm., preferably 0.5 gm., can be given once a day in the morning. The diuretic response lasts for 6 hr. or less, and during the remainder of the day disturbances in acid base balance are corrected by increased excretion of ammonia and titratable acid. Thus a response may be obtained each day under favorable circumstances, i.e., tolerance may not develop. The rationale of such treatment is the following: sodium and bicarbonate ions are excreted during the period of diuresis; chloride is excreted along with ammonia during the drug free interval. The

net effect is a modest daily loss of sodium and chloride ions although the drug per se causes loss of sodium and bicarbonate ions.

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higher, divided into 4 doses per day. As much as 8.0 gm. per day has been given for short periods with no adverse effects. However, no greater response is obtained with 8.0 gm. than with 2.0 gm. Tolerance does not develop to chlorothiazide in the same sense that it does to acetazoleamide, i.e., as a consequence of development of metabolic hyperchloremic acidosis. The reason is clear; chlorothiazide is a rather ineffective inhibitor of carbonic anhydrase and in the usual daily dose does not cause the excretion of appreciable quantities of bicarbonate. The clinical diuretic efficacy of chlorothiazide depends on its capacity to promote sodium and chloride excretion, not sodium and bicarbonate excretion. There is some *tendency for chlorothiazide to cause the development* of hypochloremic alkalosis of insignificant proportions. The alternation of courses of chlorothiazide and acetazoleamide or dichlorphenamide, the coadministration of the drugs or even the combination of chlorothiazide with small doses of ammonium chloride might be expected to correct this abnormality and render drug action more effective. Bayliss, Laragh and others recommend the coadministration of chlorothiazide and mercurial diuretics in diuretic resistant patients.

Chlorothiazide is of course as susceptible to nonacidotic mechanisms of drug refractoriness as any other diuretic. That is, low glomerular filtration rate, high rate of aldosterone secretion and hypokalemia all conspire to reduce diuretic activity.

Toxicity. The longer a drug is used, the broader becomes the spectrum of its toxic manifestations. Toxic actions of acetazoleamide are therefore recognized as more numerous than are those of chlorothiazide and dichlorphenamide, if for no other reason, because it has been used for a longer period of time.

If as much as 1.0 gm. of acetazoleamide is administered per day for any significant period of time, minor toxic manifestations are prone to develop, including anorexia, nausea, vomiting, diarrhea, paresthesias of face and extremities, lassitude, and drowsyness. Occasionally light sensitive skin rashes and rarely bone marrow depression results. Chlorothiazide, in comparison, has been described as occasionally producing gastric discomfort, mild paresthesias and skin rashes, but, in general, to be remarkably free of adverse

side reactions. Animal studies indicate that dichlorphenamide is a remarkably benign agent. However, it has not been sufficiently used in man to define its toxic manifestations when employed in diuretic therapy. All three drugs can produce potassium depletion and signs and symptoms of hypokalemia. Depletion of potassium is especially marked in patients with cirrhosis and evidence of marked liver dysfunction. Acetazoleamide and probably dichlorphenamide and chlorothiazide cause a significant increase in blood ammonia in patients with chronic liver disease. These two factors may precipitate hepatic coma in patients with impending precoma. Sherlock advises supplementation of dietary potassium in all patients with cirrhosis and ascites treated with acetazoleamide or chlorothiazide as a prophylaxis against potassium depletion and hepatic coma. Potassium supplements should also be given when chlorothiazide is used in the treatment of hypertension, for prolonged therapy with sulfonamyl compounds may cause potassium depletion in non-edematous as well as edematous patients. Digitalis toxicity is especially prone to occur in patients treated with sulfonamyl compounds. It is therefore wise to supplement potassium intake upon institution of diuretic therapy. Hyponatremia may develop as a consequence of salt restriction and excessive fluid intake and is managed as described in Chapter XIX. Daily doses of acetazoleamide may produce a marked metabolic acidosis in patients with severe renal insufficiency, a complication less apt to occur with chlorothiazide because it is less effective as an inhibitor of carbonic anhydrase. Dichlorphenamide will probably prove similar to acetazoleamide in this respect.

Comparison of Potentialities of Acetazoleamide, Dichlorphenamide, and Chlorothiazide. Acetazoleamide, dichlorphenamide, and chlorothiazide are useful drugs when properly employed in selected patients. Experience with dichlorphenamide and chlorothiazide has been insufficient to justify a truly valid comparison, although limited clinical experience indicates that the latter drug is considerably more potent and more widely effective than acetazoleamide. Acetazoleamide finds its greatest use in states of moderate salt retention, not in severely ill patients maximally retaining sodium. The nature of the disease process seems much

less significant than its severity. The need for a potent diuretic is, of course, related to intensity of salt retention, thus to severity of the disease process. However, the usefulness of an oral diuretic agent is related to its freedom from undesirable side reactions, its ability to control moderate salt retention, thus to liberalize the dietary regimen, and its capacity from day to day to maintain a stable edema free state. In certain patients, acetazoleamide accomplishes these ends admirably. General experience has been that the severely ill patient responds inadequately.

Chlorothiazide gives promise of greater efficacy in patients with more intense salt retention. Ford, Laragh, Schreiner, Bayliss and others find it effective in a variety of edematous states and in some patients refractory to other forms of diuretic therapy. Whether it will supplant mercurial diuretics, potentiated with ammonium chloride, aminophylline, and/or corticosteroids in the treatment of severely ill patients must be decided on the basis of wider clinical trial.

Certain physiological considerations incline one to favor chlorothiazide over acetazoleamide or dichlorphenamide as a diuretic apart from the ultimate criterion of clinical efficacy. A diuretic which blocks reabsorption of chloride and sodium ions should be more effective in the treatment of edema than one which blocks reabsorption of bicarbonate and sodium ions. The reasons for this are simple. (1) The filtered load of chloride is some four times the filtered load of bicarbonate. Were one to block 10 per cent of the reabsorption of chloride and sodium, four times as much edema fluid would be removed as if one were to block 10 per cent of bicarbonate reabsorption. (2) If instead one considers the effect of the urinary loss of 140 mEq. of sodium as bicarbonate, the distortion of the acid-base patern of the extracellular fluid would be greater than if the same amount of sodium were lost as chloride. (3) The distortion of the acid-base pattern produced by loss of sodium and chloride (metabolic alkalosis) can be easily corrected by administration of ammonium chloride, which in effect adds neither anion nor cation to the body fluids, but merely substitutes chloride for bicarbonate. The distortion produced by loss of sodium and bicarbonate can be corrected only by giving sodium

bicarbonate or some sodium salt with a metabolizable anion (neither procedure is desirable) or by relying on the kidneys to correct the imbalance, which they may do with little or no net loss of fixed cation.

SUMMARY

N' unsubstituted sulfonamyl compounds inhibit carbonic anhydrase and thus depress the hydration of carbon dioxide to form carbonic acid. It is probable that these drugs reduce the availability of hydrogen ions to both proximal and distal tubular ion exchange mechanisms. The proximal tubular mechanism exchanges hydrogen ions for sodium ions in the process of reabsorbing sodium bicarbonate. Enzymatic blockade of this system results in the excretion of sodium and bicarbonate ions. The distal mechanism exchanges either hydrogen or potassium ions for sodium ions in processes concerned with reabsorption of bicarbonate, with acidification of the urine, and with secretion of potassium and ammonia. Enzymatic blockade of this system results in alkalinization of the urine, in the excretion of additional sodium and bicarbonate ions, and in the secretion of potassium rather than hydrogen ions or ammonia.

Inhibition of carbonic anhydrase depends on the presence within the drug molecule of an unsubstituted sulfonamyl group, $-SO_2 \cdot NH_2$. Heterocyclic compounds, such as acetazoleamide, or halogen substituted disulfonamides, such as dichlorphamide, are most active. Sulfonamyl benzothiadiazines, such as chlorothiazide and perhaps dichlorphenamide as well possess an additional property, namely, the ability to block the proximal tubular reabsorption of sodium and chloride ions. The clinical efficacy of chlorothiazide as a diuretic depends on this latter property, its capacity to inhibit carbonic anhydrase is weak and it rarely causes excretion of bicarbonate or alkalinization of the urine when given orally to patients.

Acetazoleamide, by blocking the reabsorption of sodium bicarbonate, produces metabolic hyperchloremic acidosis. Acidosis is a major cause of the refractoriness which develops when acetazoleamide is administered in repeated doses. Chlorothiazide, by blocking the reabsorption of sodium chloride produces metabolic hypo-

chloremic alkalosis. This disturbance of acid base balance is relatively mild and does not seem to alter drug efficacy. It is readily correctible by coadministration of acetazoleamide, dichlorphenamide, or small doses of ammonium chloride. Dichlorphenamide is claimed on the basis of animal experiments to induce drug refractoriness to a lesser extent than acetazoleamide. Whether this will be borne out in clinical usage is unknown. Reduction of filtration rate and excessive secretion of aldosterone contribute to refractoriness to all sulfonamyl compounds. Mercurial diuretics may be administered in association with chlorothiazide but not with acetazoleamide. Acetazoleamide, chlorothiazide and dichlorphenamide are relatively benign and when given in proper dosage produce few side reactions. However, *all sulfonamyl compounds may cause depletion of body stores of potassium, and it is imperative that dietary intake be adequate when these drugs are administered over prolonged periods of time.*

Acetazoleamide produces adequate diuresis in mild salt retaining states but is frequently ineffective in more severely ill patients. Limited clinical experience indicates that chlorothiazide may produce diuresis in more severely ill patients. The severity of the disease process rather than its nature appears to be the limiting factor in the therapy of edema with any of these agents. Clinical usage of dichlorphenamide has not been sufficient to assess its potentialities.

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Chapter XVIII

POTASSIUM SALTS

POTASSIUM salts are among the oldest of diuretics. Thomas Willis in 1679 first recommended the administration of potassium nitrate in dropsy, and even today the nitrate is accepted as the most effective salt. The order of efficacy is claimed to be nitrate, chloride and bicarbonate, acetate, and citrate, the last three having equal diuretic potency. All of these salts may produce loss of sodium and water and decrease in body weight when administered in adequate dosage to edematous patients. Because many patients respond in an inadequate fashion, potassium salts are rarely used today as primary diuretics. However, they are valuable adjuvants in therapeutic regimens which include more potent diuretics, for all such drugs increase urinary excretion of potassium. If dietary intake is inadequate to cover urinary losses, hypokalemia and depletion of cellular stores of potassium occur. Muscular weakness, cardiac irregularities, anorexia, and abdominal distension, associated with diuretic therapy, are frequent results of depletion of potassium stores. Digitalis intoxication in the cardiac optimally digitalized prior to diuretic therapy and precipitation of hepatic coma in the cirrhotic can likewise be assigned in many instances to potassium depletion. Potassium supplementation is especially necessary when daily doses of acetazoleamide, chlorothiazide, or dichlorphenamide are administered.

Mechanism of Diuretic Action. According to Berliner, most of the potassium entering the renal tubule in the glomerular filtrate is reabsorbed in the proximal segment; that which is excreted in the urine is in large part added in the distal portion of the nephron. It is probable that some potassium is secreted in the collecting duct. As pointed out in Chapter IV, potassium ions are secreted into the urine in exchange for sodium ions by the same distal

mechanism which secretes hydrogen ions. When potassium chloride is administered in adequate dosage, the urine becomes alkaline and contains increased amounts of potassum, bicarbonate and sodium. Chloride excretion is only slightly increased. If potassium nitrate or sulfate are administered, somewhat more chloride is eliminated.

These findings are most adequately explained by the now generaly accepted thesis that hydrogen and potassium ions compete for a common distal transport mechanism. If large numbers of potassium ions are supplied to the exchange mechanism, potassium rather than hydrogen is transported, and the urine becomes alkaline. According to Berliner, Kennedy and Orloff, competition between potassium and hydrogen ions for the common exchange mechanism is not on a one to one basis; instead it is roughly on a two to three basis. Thus for the secretion of every two potassium ions, three hydrogen ions are displaced from the exchange mechanism. One less sodium ion and three fewer bicarbonate ions are reabsorbed. There results a net loss of one sodium ion from body stores for each two potassium ions secreted. The excess cations are largely eliminated as bicarbonate. Because hydrogen ion exchange is depressed and the urine becomes alkaline, ammonia excretion declines. Continued oral administration of potassium salts therefore results in the development of a mild hyperchloremic metabolic acidosis. The acidosis is self-limited, for in the course of a few days chloride replaces bicarbonate in the urine. The diuretic action of potassium salts is also evanescent; within a few days sodium loss in the urine ceases.

Potasium salts also act as osmotic diuretics (*cf.* Chapter XIV). Such efficacy as they may have in this respect is due to their rapid elimination by tubular secretion. In the postabsorptive state, the potassium clearance of the normal individual is less than one-quarter the rate of glomerular filtration, a fact which indicates extensive tubular reabsorption. Following ingestion of a potassium salt, clearance rises sharply with relatively little increase in serum concentration to approach and even to exceed glomerular filtration.

Dosage. Potassium salts have been administered orally in daily doses of 5 to 10 gm. of the chloride; 8 to 12 gm. of the nitrate,

and up to 20 gm. of the citrate. They may be given in divided doses 3 to 4 times daily in 10 per cent solution or in capsules. In general, the greater the dosage, the more adequate the response, but at best results are not impressive. Within the dose range noted above, potassium salts are well tolerated by most patients, and cause only minimal changes in serum concentration. This latter finding results from the fact that potassium penetrates cells readily, hence distributes throughout total body water; intracellular concentration increases in proportion to the increase in extracellular concentration. However, in some patients, potassium salts cause gastric irritation. Because of their brief diuretic action, potassium salts are commonly administered for 3 to 4 days, followed by a rest period of equal length. When given prophylactically, they are administered daily.

Contraindications. Potassium salts should not be given to patients with markedly impaired renal function and elevated serum potassium levels, because of the danger of potassium intoxication with its manifestations of cardiac conduction disturbances and cardiac arrest. Renal disease per se is not a contraindication to use of potassium salts for the capacity to excrete potassium is well maintained until late in the disease process.

SUMMARY

Potassium salts are weak diuretics by virtue of the fact that the exchange of hydrogen ions for sodium ions in the distal portion of the nephron is depressed to a greater degree than the exchange of potassium ions for sodium ions is enhanced. They also act as mild osmotic diuretics. The major use of potassium salts in diuretic therapy is in prophylaxis of hypokalemia and depletion of cellular potassium reserves.

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Chapter XIX

HYPONATREMIA AND POTASSIUM DEFICIENCY

REDUCTION of the sodium concentration of extracellular fluid (hyponatremia) and depletion of body reserves of potassium (associated with hypokalemia) are complications which may arise in the course of therapy with any effective diuretic agent. These alterations in composition of the body fluids increase disability, interfere with reduction of edema, and when severe, jeopardize life. Within limits, they can and should be avoided; if they occur, they should be recognized and treated appropriately.

HYPONATREMIA

Hyponatremia in clinical parlance means a reduction in the plasma or serum concentration of sodium to a value less than 135 mEq. per liter, the lower limit of normal, although the term literally means a reduction in the sodium concentration of blood. Actually hyponatremia is a clinically significant cause of symptoms and signs (low salt syndrome) only when concentration falls below 125 mEq. per liter. Furthermore, as Albrink et al have pointed out, some degree of hyponatremia is entirely normal in marked hyperlipemia. Lipid occupies volume in plasma, sodium is dissolved in water. Accordingly, the sodium concentration in the aqueous phase of plasma, hence in extracellular fluid, may be normal, yet concentration per liter of plasma may be subnormal in proportion to volume occupied by lipid. Even in true hyponatremia, symptoms and signs referable to this abnormality are conditioned both by the rate at which it develops and by its inciting cause. We shall confine this discussion to hyponatremia associated with edema and/or ascites which develops in the course of and often as a consequence of therapy. Welt distinguishes three types of hypona-

tremia, chronic hyponatremia, acute hyponatremia without evidence of reduction in plasma volume, and acute hyponatrema with evidence of peripheral circulatory failure. All are fundamentally dilutional in origin, i.e., water is retained in the body in excess of sodium, or conversely sodium is excreted in excess of water.

Chronic Hyponatremia develops gradually and is most frequently observed in nephrosis, cirrhosis with ascites, and in severe and long standing congestive circulatory failure. It also occurs late in the course of many severe debilitating diseases unassociated with edema, e.g., advanced pulmonary tuberculosis and generalized carcinomatosis. It may be an expression of wide spread depression of metabolic functions of all cells. Since cachexia is often a common factor, it has been loosely termed the "sick cell" syndrome. Chronic hyponatremia per se is singularly asymptomatic. Little evidence of cellular edema exists and attempts to correct the obvious hypoosmolality of extracellular fluid by the infusion of hypertonic sodium chloride causes intense thirst; when water is ingested, body fluids are rediluted. Those patients unable to excrete a salt load, i.e., those with edema or ascites, expand extracellular and transcellular fluid volumes. Those patients capable of excreting a salt load do so and return to their pretreatment condition. In any event chronic hyponatremia is a grave sign.

The cause of chronic hyponatremia is not known with any certainty. However, one may speculate that it represents a primary reduction in osmolality of cells and an associated secondary reduction in osmolality of extracellular fluid. Presumably the osmotic activity of the contents of the intracranial osmoreceptors, like that of other cells, is reduced. Therefore, they respond to concentration and dilution of extracellular fluid in an abnormal fashion; i.e., the osmostat (analogous to a thermostat) is reset to a lower level.

Laragh has shown that the oral administration of 4 to 22 gm. of KCl to chronically hyponatremic edematous patients may cause a striking increase in the extracellular sodium concentration without the administration of exogenous sodium and without loss of water. Potassium enters cells in exchange for sodium. If more potassium enters than sodium leaves, cellular osmolality increases, and water

enters by osmosis. The transfer of sodium and possibly hydrogen ions to extracellular fluid in exchange for potassium and the migration of extracellular water into cells both conspire to increase extracellular osmolality and to raise plasma sodium concentration How universally potassium therapy will correct hyponatremia is not known. The procedure is not without hazard for it appears that the chronically edematous hyponatremic patient is less capable of excreting an excessively large potassium load than is the non-hyponatremic patient. Elevation of plasma potassium is therefore prone to occur.

Chronic hyponatremia is commonly associated with resistance to diuretic therapy, the usual regimens causing neither loss of water nor loss of sodium. As was pointed out in Chapter XII, Schemn first observed that certain edematous, hyponatremic, and moribund patients, resistant to all common forms of therapy, could be made responsive by treatment with ACTH and cortisone. Subsequently others showed that prednisone and prednisolone were also effective in potentiating diuretic activity. Often steroids first induce water diuresis, restoring osmolality toward normal, then in combination with organomercurial or sulfonamyl compounds induce sodium and water diuresis, causing further reduction of edema. It is not known if the primary action of steroids is to promote the excretion of water by a direct renal action, resulting secondarily in an increase in extracellular and cellular osmolality, or if their primary action is to increase cellular osmolality, including a resetting of the osmostat, resulting secondarily in a loss of water from the extracellular compartment.

As has been pointed out in preceding chapters, various procedures may be employed to increase diuretic efficacy in resistant patients, including those with chronic hyponatremia. However, addition of salt to the diet or the infusion of hypertonic saline is relatively ineffective either in correcting hyponatremia or in restoring responsiveness to diuretics.

Acute Dilutional Hyponatremia Without Reduction in Plasma Volume commonly develops at an intermediate rate in patients maintained on a low salt diet, subjected to intensive diuretic therapy, and encouraged to drink excessive amounts of water.

Except for the presence of edema, the condition is akin to that which develops in normal subjects maintained on a low salt intake, depleted of sodium by profuse sweating, and allowed free access to water, as described by McCance and others. Hemoconcentration and signs of peripheral circulatory failure are absent, but the patient is restless, complains of muscle cramps, exhibits muscle twitching, becomes lethargic or comatose, and finally convulses. Symptoms and signs are those of water intoxication and in large part may be assigned to cellular edema. Superimposed on this picture may be evidence of progressive renal failure, with reduced glomerular filtration rate and elevated blood urea nitrogen.

It was pointed out in Chapter V that osmolality and volume of extracellular fluid are regulated by semi-independent mechanisms. However, it was further stated that interaction occurs and that precise regulation of osmolality may be sacrificed in the interest of partial maintenance of volume. The patient with an absolute or relative reduction in plasma or extracellular fluid volume exhibits thirst, drinks water and partially restores volume at the expense of reduced extracellular and cellular osmolality. Two possible causes of water retention have been suggested. First, the volume receptor mechanism acting through the osmoregulatory-antidiuretic hormone mechanism, stimulates retention of water, even though osmolality of the body fluids is depressed. Second, the volume receptor mechanism, acting through autonomic or humoral effector mechanisms, reduces glomerular filtration rate. As Berliner and others have shown, a reduction of filtration rate of sufficient magnitude will cause over-reabsorption of water and formation of small volumes of hypertonic urine, even though the body fluids are excessively diluted. It is not known which of the two mechanisms is the more significant; both might well play a role.

If the symptoms and signs of acute dilutional hyponatremia are mild, withholding of water, cessation of diuretic therapy, and liberalization of salt intake may be all that is required. If moderate, hypertonic urea may be given by gavage to abstract water from the body osmotically. If severe, hypertonic saline, given intra-

venously, is necessary and often provides immediate and dramatic relief.

Acute Dilutional Hyponatremia With Evidence of Peripheral Circulatory Failure develops shortly after massive paracentesis, profound diuresis, and severe vomiting or diarrhea. The clinical picture resembles that described above but includes in addition, symptoms and signs of peripheral circulatory collapse. Fall in blood pressure, rapid, weak, thready pulse, cold, clammy cyanotic skin, oliguria or anuria, and hemoconcentration are added to the signs of water intoxication.

The removal of large volumes of ascitic fluid reduces intra-abdominal pressure. Transudation occurs at an accelerated rate and plasma volume is reduced. Thirst drives the patient to replace his fluid deficit, but most of the water ingested enters cells, interstitial fluid, and the peritoneal cavity. Body fluids are diluted, only a small fraction of the water is retained in the vascular bed, and peripheral circulatory failure results. Massive diuresis, vomiting, or diarrhea may similarly result in reduced circulating blood volume, thirst and dilution of body fluids. The factors which limit the excretion of water and restoration of osmolality of body fluids are those described above. Treatment is that for acute dilutional hyponatremia without evidence of reduction in plasma volume.

The occurence of acute dilutional hyponatremia with or without evidence of peripheral circulatory collapse can frequently be avoided by repeated paracentesis of small volumes of fluid (2 or 3 liters) rather than by a single massive abdominal tap. Similarly, diuretics should be administered to massively edematous patients in such amounts as to remove not more than 2 to 3 liters of edema fluid per day. The more profound the diuresis, the greater is the incidence of complications.

Hypertonic Saline in the Treatment of Hyponatremia in edematous patients should be employed with caution and only in the presence of symptoms and signs of frank water intoxication: lethargy, coma, muscle twitching, or convulsions. In less critical situations, the withholding of fluids, cessation of diuretic therapy, and liberalization of salt intake will ameliorate the condition.

According to Welt the quantity of sodium required to restore

osmolality of the body fluids to normal is equal to the deficit in concentration per liter of plasma multiplied by the estimated number of liters of total body water. This does not imply, as one might surmize, that sodium is distributed throughout body water, cellular as well as extracellular. Rather it implies a uniform osmolality throughout all body fluids which demands that an increase in the concentration of sodium in extracellular fluid be accomplished by the same increase in total solute concentration in the cell water. This increase in intracellular concentration will be accomplished by the movement of water from the cells in response to the addition of hypertonic salt to the extracellular fluid. Although the administered salt will, for the most part, be confined to the extracellular compartment, its osmotic effect is distributed throughout both compartments. In terms of mEq., it is advisable to administer one-fifth of the dose as sodium bicarbonate, four-fifths as sodium chloride to avoid acidosis. It must be given intravenously at a slow rate in hypertonic solution, 3 to 5 per cent, and water must be withheld for the succeeding 6 to 12 hr. to prevent redilution and increased edema. It is preferable to give only a part of the calculated dose and to note the response. Marked clinical improvement will occur with partial correction of hyponatremia if symptoms and signs are in truth due to this abnormality of body fluid composition.

HYPOKALEMIA AND POTASSIUM DEFICIENCY

Depletion of body stores of potassium is also a potential complication of all forms of diuretic therapy. It is most prone to develop in the course of prolonged or intensive treatment with mercurial diuretics, hydrogen and ammonium cycle resins, ammonium chloride, and sulfonamyl compounds, especially if anorexia or vomiting limit dietary intake or if diarrhea causes increased fecal loss. Under most circumstances, hypokalemia, i.e., reduction of the plasma concentration of potassium to a value less than 3.5 mEq. per liter, is indicative of potassium depletion. However, the extent of the reduction in plasma potassium is no adequate gauge of the magnitude of total body deficit.

Manifestations of Potassium Depletion include the neuromuscular signs of diminished deep reflexes, weakness progressing to flaccid paralysis, and mental confusion; the gastrointestinal signs of anorexia, diarrhea, abdominal distension, and ultimately paralytic ileus; the cardiac signs of rapid rate and irregular rhythm; and the renal signs of isosthenuria, and less frequently, reduction in filtration rate and azotemia.

Potassium depleted patients are highly sensitive to the digitalis glycosides. Those who are on maintenance doses and who are adequately digitalized show signs of toxicity if potassium stores are even modestly depleted. Electrocardiographic abnormalities of potassium depletion and/or digitalis intoxication include low or inverted and broadened T waves, prolongation of the Q-T interval, and sagging and finally depression of the S-T segment. Increased excitability and abnormalities of impulse initiation and conduction may become evident Potassium ions antagonize the effects of digitalis glycosides on the heart; calcuim ions potentiate them. Therefore either potassium depletion or calcium excess will induce digitalis toxicity.

Isosthenuria, polyuria, and insensitivity to ADH are the major functional manifestations of renal potassium depletion and are more or less reversible with therapy. Potassium depleted kidneys seem especially vulnerable to the development of pyelonephritis Reduced glomerular filtration rate, azotemia and even acute renal failure have been described as resulting from potassium depletion and as responding favorably to potassium repletion.

In animals experimentally depleted of potassium, lesions have been described in skeletal muscle, cardiac muscle, and the Purkinje system. Muscle fibers exhibit edema and fragment, focal areas of necrosis develop, and the degenerated regions become fibrotic. Fibers of the Purkinje system show granulation and vacuolation. Somewhat similar lesions have been observed in patients who succumb in diabetic ketosis, diarrheal diseases, and other conditions associated with potassium depletion Renal lesions are confined mainly to the tubular epithelium and include swelling, hyperplasia, granulation, and vacuolation. Changes are most marked and appear earlier in the collecting ducts. In the experimental

animal, swelling and hyperplasia of clear cells and proliferation of intercalated cells may obstruct collecting ducts and lead to dilation of more proximal portions of the nephron. Cytological changes are observed in the proximal and distal tubules in long standing potassium depletion. Despite the functional derangements of the gastrointestinal tract and central nervous system, no striking pathological changes occur in gut, brain or spinal cord.

In severe hepatic insufficiency the capacity of the liver to remove ammonium ion from the portal blood is reduced and accordingly the concentration of this ion in systemic blood increases. In hypokalemia the partial pressure of free ammonia (PNH_3) rises due to the associated alkalosis. Apparently toxicity is more related to PNH_3 than to total ammonia plus ammonium ion concentration, for cells are far more permeable to free ammonia than to the ion. Potassium deficiency is a predisposing cause of hepatic coma in liver disease and its deleterious effect is in part due to alkalosis and increased PNH_3.

Maintenance of Normal Intracellular Potassium Ion Concentrations depends on the continuous active transport of potassium into cells and the continuous active extrusion of sodium from cells, processes discussed in some detail in Chapter II. If potassium is lost from extracellular fluid into urine, vomitus, or feces, the normal steady state relationship is disturbed As Darrow and others have shown, potassium ions move out of cells to replace extracellular losses and sodium and hydrogen ions enter cells These ion shifts result in extracellular hypokalemic alkalosis and intracellular acidosis If on the other hand an acid load is imposed on the body, the acid is buffered in part by the entry of hydrogen ions into cells in exchange for potassium ions. The potassium lost by cells is excreted in the urine.

Depletion of Cellular Stores of Potassium in the Course of Diuretic Therapy may be explained in terms of one of the other of the two mechanisms outlined above. Ammonium or hydrogen cycle resins have a greater affinity for potassium than for sodium. As pointed out in Chapter X, they bind significant quantities of potassium in the gut. Furthermore, the colonic mucosa of edematous patients actively conserving sodium is stimulated by the high

titre of circulating aldosterone to exchange potassium derived from extracellular fluid for fecal sodium bound to resin. Potassium lost in the feces is replaced from cellular stores. To prevent progressive depletion of cellular potassium, at least one-third of the dose of resin is commonly administered in the potassium cycle.

Ammonium chloride is converted in the liver to urea and hydrochloric acid. As pointed out in Chapter XI, an appreciable fraction of this acid is neutralized by intracellular buffers, hydrogen ions entering cells in exchange for potassium ions. The potassium is excreted in the urine to maintain extracellular concentration within the narrow limits of normal. Accordingly, cellular stores are reduced, and if dietary intake is inadequate, serious depletion may result.

Mercurial diuretics inhibit a limited fraction of the proximal tubular reabsorption of sodium and chloride ions. In edematous patients, circulating aldosterone stimulates the ion exchange mechanisms located in the distal tubule and collecting ducts. The excess sodium which enters the terminal part of the nephron from the proximal segment may be exchanged in large part for potassium, hydrogen and ammonium ions. The chloride is excreted in association with these ions rather than sodium. Osmotic diuretics, which interfere with proximal reabsorption of sodium and chloride ions, may likewise induce the excretion of potassium because of enhanced distal exchange.

The sulfonamyl inhibitors of carbonic anhydrase more directly promote potassium loss by reducing the availability of hydrogen ions to the exchange mechanism. Blockade of exchange of hydrogen and ammonium ions for sodium ions is compensated by increased exchange of potassium ions. The more intense the stimulation of the exchange mechanism by circulating aldosterone, the greater is the depletion of body stores of potassium.

Treatment and Prophylaxis of Potassium Deficiency. The extent of depletion of postassium stores cannot be estimated in any way applicable to the routine management of patients. It can be measured in the course of balance studies by determining the quantity of potassium retained when daily oral supplements are administered over a prolonged period of time. It may also be

estimated by isotope dilution methods. There is, however, no need for exact knowledge of deficits; if any of the symptoms or signs of potassium depletion appear, supplementation of intake with 250 ml. or more per day of orange juice or 3 to 5 gm. per day of KCl will induce a remission of symptoms well before the deficit is corrected. The correction of large deficits, of the order of 400 *to 1000 mEq. must be done slowly over a period of weeks.* Prevention of potassium depletion by insuring adequate dietary intake is far preferable to treating deficiency when it occurs. Although potassium salts per se are not especially potent diuretics, their routine use along with more effective agents does no harm and guards against the development of deficits. Whenever sulfonamyl diuretics are used, potassium supplements should be given. This is equally true in the use of these agents in hypertensive therapy for sizable doses are administered daily over prolonged periods of time. Only in severe renal disease with basally elevated plasma potassium levels should potassium salts not be used. Under such conditions, there is usually no cause for diuretic therapy of any sort.

SUMMARY

Hyponatremia and depletion of body stores of potassium may occur in the course of therapy with any effective diuretic agent. These alterations in ionic composition of the body increase disability, render treatment ineffective, and when severe, jeopardize life.

Acute hyponatremia may develop rapidly in response to massive paracentesis or profound diuresis or at an intermediate rate in the course of intensive diuretic therapy. It results from stimulation of antidiuretic mechanisms by reduction in circulating blood volume, and may be associated with signs of peripheral circulatory collapse. Thirst, ingestion of water, and inhibition of water diuresis cause dilution of body fluids. The condition may be avoided by less intensive diuretic therapy and by repeated abdominal taps of small volume rather than a single massive tap. Mild to moderate hyponatremia can be adequately managed by withholding water, stopping diuretic treatment, and liberalizing salt intake. Only if signs of water intoxication are severe should hypertonic saline be

given. Chronic hyponatremia develops gradually late in the course of severe congestive failure, nephrosis and cirrhosis with ascites. In this form, hyponatremia is resistant to therapy and a grave prognostic sign.

Depletion of body stores of potassium during diuretic therapy is a consequence either of primary loss of extracellular potassium in feces or urine with replacement from cells, or of primary loss from cells with increased urinary excretion to stabilize plasma concentration. The former is the more common mechanism. Thus cation exchange resins increase fecal excretion of potassium. Mercurial diuretics, osmotic diuretics and sulfonamyl compounds increase urinary excretion of potassium. Fecal and urinary excretion result secondarily in cellular loss of potassium and gain of sodium and hydrogen ions. Acidifying agents such as ammonium chloride cause primary loss of cellular potassium, hydrogen ions entering cells in exchange for potassium. An increase in extracellular potassium results in increased urinary excretion.

Potassium depletion causes symptoms and signs referable to the neuromuscular, gastrointestinal, circulatory and excretory systems It is more apt to occur if diuretic therapy is intensive and if dietary intake of potassium is inadequate. Whenever diuretics are administered over any extended period of time, the adequacy of potassium intake must be assured.

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